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THE UNIVERSITY OF ALBERTA

AN AUTORADIOGRAPHIC STUDY OF PERIODONTAL DEVELOPMENT

by

DOUGLAS G.S. PRICE



A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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THE UNIVERSITY OF ALBERTA
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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled AN AUTORADIOGRAPHIC STUDY OF PERIODONTAL DEVELOPMENT submitted by DOUGLAS G.S. PRICE in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE in ORAL BIOLOGY.

Date... *Dec 4, 1984*

DEDICATION

This thesis is dedicated to my wife Amanda who, with her encouragement, understanding and patience, made the completion of this work possible, and to my children Laura and Alexander.

ABSTRACT

It has long been accepted that the periodontium (with the possible exception of the gingiva) develops from the dental follicle. Results from a number of transplantation studies led Ten Cate (1969, 1972) to propose that the investing layer of the dental follicle acts as the prime source of most of the periodontal tissues. This view has subsequently become generally accepted.

More recently however, there have been suggestions that periodontal development is rather more involved. In particular the results of a study by Yoshikawa and Kollar (1981), in which components of tooth germs were separated then recombined and cultured intraocularly, provided firm evidence that the dental papilla could also be important in the development of the periodontium. When considering the evolution and development of different forms of tooth attachment, Osborn (1984), in an extension of his cladistic theory of morphogenesis (1978a,b), proposed that cells migrate from the dental papilla into the follicle during tooth development and are important later in the formation of the periodontium. The presence of periodontal tissues within the pulp in certain pathologies and observed in the studies of Hoffman (1960, 1966) appear to support the hypothesis. It is the purpose of this study to test Osborn's hypothesis that cells move from the papilla into the follicle during tooth development.

The mandibular third molar of a strain of albino mouse has been studied using a method similar to that employed by Perera and Tonge (1981a) and McCulloch and Melcher (1983). An intra-peritoneal injection of tritiated thymidine was given to 10 day and 13 day old mice. The labelling index within defined areas of the dental papilla and follicle of M₃ at set times after injection was calculated. It was possible to determine if cells moved between areas by examining changes in the labelling index with time.

From the results it was concluded that cells move from the papilla to the follicle in the 10 day old mice but movement ceases by 13 days. In addition there appeared to be an area of proliferation within the dental follicle just lateral to the cervical loop. This area received, or acted as a highway for, cells from the papilla in the 10 day old animal but had become

independent of the papilla by 13 days.

The vital role played by the follicle in periodontal development is not denied. It appears, however, that the dental papilla is also important. It is proposed that normal periodontal development is the result of the dental papilla and follicle acting in concert.

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1. INTRODUCTION

Teeth are adapted to process the food which an animal eats. But no matter how perfect their structure and morphology they would be useless without an adequate supporting mechanism.

1.1 Tooth Attachment

It is possible to divide the forms of tooth attachment into two groups. Teeth can be rigidly attached to the jaw bone by means of mineralized tissue (ankylosis) or by a deformable fibrous attachment (gomphosis) in which unmineralized fibres connect the root to the bone of the jaw or, in Chondrichthyes, the jaw cartilages.

Ankyloses may be classified by considering the tooth position with respect to the jaw bone. In an acrodont ankylosis the tooth is cemented to the horizontal surface of the jaw bone and in a pleurodont ankylosis it is cemented to the lingual side of bone (Fig. 1A,B). A further modification, where the tooth root is surrounded by bone is called a thecodont ankylosis (Scott and Symons, 1974; Osborn, 1984) (Fig. 1 C).

With the exception of crocodiles and alligators, an ankylosis (in some form) is the most frequent type of tooth attachment in the reptiles whilst all mammals employ a gomphosis. Reptiles (such as the Crocodilia) employing a gomphosis, have what is called a thecodont gomphosis, in which a successional tooth develops within, and erupts out of the socket of its predecessor (Hopewell-Smith, 1918; Berkovitz and Sloan, 1979). In the mammalian gomphosis (Fig. 2) successional teeth develop within crypts separate from their predecessors, and their eruption leads to resorption of the predecessor and probably even its socket walls. The fibrous tissue connecting the root to the bone is called the periodontal ligament.

The change from the reptilian tooth attachment mechanism (ankylosis) to the gomphosis with concomitant lengthening and tapering of roots, presumably conferred an advantage to mammals. One advantage is that the gomphosis allows tooth movement which can compensate for changes due to growth of the jaws or wear of the teeth and thereby maintain the

functional efficiency of the teeth (Gottlieb et al, 1938). With an ankylosis the only way the position of a functioning tooth can be changed is by removing it and erupting a replacement tooth in the new, more advantageous, position.

Lengthening of mammalian tooth roots results in an increase in the strength of attachment and tapering, with closure of the apex (Fig. 3), lessens the chance of root fracture. A further important advantage conferred by a gomphosis is its ability to act as a stress-breaker, protecting the tooth from forces which might fracture it. The disruptive forces of mastication used by mammals to break up food in the mouth might necessitate a stress-breaker. This is not necessary for reptiles because they only use their teeth to grasp food which is then swallowed without being processed by the teeth.

1.2 Normal Anatomy of The Periodontium

The tissues involved in a mammalian tooth attachment are collectively termed the periodontium and consist of alveolar bone, periodontal ligament, cement and the gingival tissues (Fig. 4). Although the periodontium may be regarded as a functional unit it is convenient to consider each component of the periodontium in turn.

1.2.1 The Periodontal Ligament

The periodontal ligament is the unmineralized connective tissue which lies between the root of the tooth and the surrounding alveolar bone. Its width varies (Kronfeld, 1931; Coolidge, 1937) depending on age and the load carried by the tooth. The average width is about 0.2 mm in man but may decrease to 0.1 mm with age or lack of function, and increase to 0.3 mm in teeth which are in heavy use. The ligament width may also be decreased at the fulcrum of the tooth — the point about which the tooth rotates when subjected to tilting forces and which is usually about one third along the length of the root from its apex. The quality and organization of the fibre bundles of the ligament may also be affected by function. Increased function causes an increasingly regular organization and disuse leads to atrophy and disorganization (Coolidge,

1937).

In common with the connective tissues the periodontal ligament is composed of cells, fibres and a gel-like extracellular matrix. Histologically, collagen fibres appear the most conspicuous constituent of the ligament, being arranged in densely ordered bundles which appear to extend between cement (lining the root of the tooth) and alveolar bone (Fig. 4). The ends of the fibres embedded in cement and alveolar bone are called Sharpey fibres.

The bundles of fibres are arranged in groups (Fig. 5), the orientation of the groups varying in different parts of the root. These groups are termed the principal fibres of the periodontal ligament and can be classified according to their position and orientation (Berkovitz, 1981). The major group comprises the oblique fibres which pass from the root surface obliquely upwards and outwards, suspending the tooth in its socket and resisting axial loading. Other fibre groups resist rotation and tilting forces.

There is a constant turnover and remodelling of collagen within the ligament. Studies have indicated (Eccles, 1959; Grant and Bernick, 1972) that the principal fibres are orientated during root development and remodelled during tooth eruption.

The gel of the extracellular matrix is an important component of the periodontal ligament. It is mainly composed of proteoglycans and water with lesser amounts of carbohydrates, lipids, proteins and glycoproteins (Berkovitz, 1981). The possible functions of the gel are poorly understood but its hydrodynamic properties have been suggested to play a role in tooth support (Picton, 1969).

The majority of cells within the periodontal ligament are fibroblasts and they are responsible for the production, maintenance and remodelling of the collagen fibres and extracellular matrix. In the developing and erupting tooth the major sites of cell division within the ligament seem to be just lateral to the cervical loop (Fig. 6) (Hoffman & Gillette, 1964; Ten Cate, 1972; Perera & Tonge, 1981a). Daughter cells may be considered to either migrate coronally or to be "played out" from the proliferative area as the tooth crown moves up and out of its socket (Fig. 6). In mature erupted teeth the central part of the ligament seems to act as

the proliferative area, and daughter cells now migrate peripherally towards the cement or bone (McCulloch and Melcher, 1983). Higher cell densities are reported adjacent to cement and bone and presumably a proportion of these cells must be considered to be precementoblasts and preosteoblasts.

A network of epithelial cells — the epithelial cell rests of Malassez — exists around the root of the tooth. They are the remnants of Hertwig's root sheath, a structure which moulds the shape of the developing root, and are resting cells which have retained their ability to divide and may occasionally proliferate to form the lining of a dental cyst (Fig. 7). Other cells in the periodontal ligament are concerned with neural (e.g. end organs) and vascular functions.

1.2.2 Alveolar Bone

Alveolar bone is defined as the bone immediately surrounding the crypts of developing teeth and the sockets of erupting teeth — both in the mandible and maxilla (Widdowson, 1939). The remaining bone of the mandible and maxilla is described as basal bone (Fig. 8). However, this arbitrary division between alveolar and basal bone cannot be recognized histologically. The alveolar bone and basal bone are indistinguishable, with the exception of the layer of alveolar bone making up the walls of the socket into which Sharpey fibres are embedded (Fig. 9). By definition the presence of alveolar bone requires the presence of teeth. This seems to be the case because alveolar bone does not form in cases of anodontia (Shafer et al, 1967) and is generally lost after teeth are extracted.

1.2.3 Cement

Cement is a calcified tissue, usually regarded as a modified form of bone, which covers the roots of teeth and by means of Sharpey fibres provides an anchorage point for the principal fibres of the periodontal ligament. As a result it links the tooth via the ligament with adjacent teeth, gingiva and alveolar bone.

1.2.4 Gingiva

The final component of the periodontium — the gingiva — is that part of the periodontium which provides the seal between the periodontal ligament and the oral environment (Fig. 9). Morphologically it can be described as being made up apically of an epithelium — the junctional epithelium — which is attached to the enamel surface by means of hemidesmosomes and a basal lamina and is known as the epithelial attachment. The epithelium lining the more coronal aspect of the gingival sulcus is not attached to the enamel and is termed the oral sulcular epithelium. The epithelium then folds over thus forming the free gingiva and becomes the attached gingiva as it is fixed to the alveolar bone by its underlying connective tissue (Fig. 10).

1.3 Function of The Periodontium

It is possible to divide the functions of the periodontal ligament into supportive and sensory.

1.3.1 Supportive Function

The periodontal ligament is of vital importance in tooth support. The mechanism by which this support is provided is generally considered to be a combination of a hydrodynamic system and suspensory system provided by the collagenous principal fibres (Orban, 1928; Picton, 1969).

It has long been believed that the oblique fibres of the ligament suspend the tooth in its socket, uniting the root to alveolar bone. Under axial loading these fibres transmit pressure applied to the tooth as a tension to the socket wall (Scott and Symons, 1974). The force has also been thought to be absorbed by a straightening of the wavy course followed by the collagen fibres.

Although the principal fibres play a role in tooth support it is now thought that a hydraulic system is also extremely important (Picton, 1969). Currently it is believed that the

hydraulic system is important in resisting the transient forces produced by mastication, but prolonged forces, such as those applied orthodontically, result in remodelling of the fibre bundles.

The periodontal ligament has been intensively studied with regard to tooth eruption. The forces which cause eruption are still not known but fibroblast migration in the ligament (Ness, 1967; Zajicek, 1974) and effects of fluid pressure (Main and Adams, 1966) have been cited as providing an eruptive force, together with other theories (Osborn and Ten Cate, 1983).

1.3.2 Sensory Function

The periodontal ligament is well supplied with nerve fibres, which enter the ligament both apically and laterally through foramina in the lamina dura. Some of these fibres terminate as free unmyelinated nerve endings some of which are associated with pain reception. Other fibres are mechanoreceptors which are specifically involved in proprioception — as the proverbial grain of sand in a sandwich testifies (Hannam, 1982).

1.4 Development of The Periodontium

Periodontal development is closely associated with the presence of the dental follicle. Descriptions and definitions of the dental follicle vary from author to author (Ten Cate, 1969). For instance Tonge (1963) and Berkovitz (1981) consider the dental follicle to be all the tissue between the tooth germ and alveolar bone although describing it as being in three layers, and Fearnhead (1961) has a similar definition but describes only two layers. The classification employed by Ten Cate (1969) seems to be most appropriate in view of the role played by the follicle during periodontal development. He suggests that during early development the tissue between the tooth germ and alveolar bone should be considered as two layers. The inner, cell dense layer in immediate contact with the tooth germ being termed the investing layer, and the remaining less dense tissue the perifollicular mesenchyme (Fig. 11).

It is widely believed that the periodontium (with the exception of the gingival component) is derived from the investing layer (Ten Cate, 1969, 1972; Osborn and Ten Cate, 1983). The experimental evidence supporting such a belief is strong and its major proponent is Ten Cate (1969, 1972).

The theory was reinforced as a result of an experiment (Ten Cate et al, 1971) in which first molar tooth germs and investing layer were dissected from day old mice, labelled with tritiated thymidine, and then implanted subcutaneously in syngeneic adult hosts (Fig. 12). Examination of these grafts 3-4 weeks later revealed continued tooth development, with cement, ligament and bone developing in relation to the roots. The cementoblasts and fibroblasts of the ligament were found to be labelled and thus derived from the implant. No labelling was seen in osteocytes. However a later ultrastructural study (Ten Cate & Mills, 1972) showed the presence of a few lymphocytes external to the bone and this was taken as being an indication that the graft was being rejected because the bone was of donor origin.

Later work (Freeman et al, 1975) gave additional support. In this study labelled mouse tooth germs and adherent investing layer were implanted into holes drilled into the parietal bones of syngeneic adult hosts [such holes in the parietal bone normally become filled with fibrous tissue and not bone (Melcher, 1969)]. However, in this study continued tooth development resulted in the formation of a complete gomphosis — with cement, ligament and bone. Again the cementoblasts and ligament fibroblasts were labelled.

Ten Cate (1969) cites as further evidence the *in vitro* study of Main (1966) in which tooth germs were kept in long-term culture for 30 days. During this time they became totally disorganized and survived only as scattered epithelial and ectomesenchymal cells. These cells, harvested and placed in the base of a mouse tail tendon, reorganized into a tooth germ eventually forming teeth with associated cement, periodontal ligament and bone. In addition, intraocular grafts (a location in which osteogenic cells do not exist) of tooth germs result in continued tooth and periodontal development (Lumsden, 1977; Yoshikawa & Kollar, 1981).

In discussing such results, Ten Cate (1972) came to the conclusion that the periodontium (with the possible exception of the gingival component) is derived from the cells of the investing layer. But there were discrepancies between this theory and results obtained by Hoffman (1960) who performed subcutaneous implants of hamster molar tooth germs into syngeneic hosts which resulted in tooth, root and periodontal development — after carefully removing as much of the follicle as possible from the tooth germs. Hoffman thought the formation of periodontal ligament and bone resulted from an inductive influence exerted by the implanted tooth germ on the surrounding host tissue. However, Ten Cate explained this by referring to the few follicular cells which Hoffman mentions he was unable to dissect from the tooth germ. It is the subsequent proliferation of these few adherent follicular cells which Ten Cate believes produced the periodontal ligament and bone.

Ten Cate's theory is well-reasoned and argued. There is little doubt that the investing layer is of prime importance in periodontal development. However, when reviewing the theory, the impression is given that the investing layer exists as a separate tissue. But examination of photomicrographs in articles (Ten Cate, 1969) and descriptions (Scott & Symons, 1974) show that in fact there is a continuity between the dental papilla and investing layer (Fig. 13).

The question then arises — can the cells of the dental papilla contribute to periodontal development by migrating out of the papilla into the investing layer? The possibility of such a migration was postulated by Osborn (1984) in an extension of his cladistic interpretation of development. The basic premise of Osborn's (1978a,b) cladistic theory is that a cell is sequentially bipotent rather than being multipotent (Dienstman and Holtzer, 1974). Therefore upon division cells may enter one of two differentiation compartments — which he terms clades. Using such a system "family trees" of cell lineages can be constructed, called cladograms. The cladograms predict the existence of intermediate cell populations, or clades, which are necessary in order to produce the diverse, fully differentiated cell types which are required for tooth and periodontal development.

The cladogram (Fig. 14) is taken from Osborn (1984) and represents a hypothetical lineage from one clade of cells — the primitive tooth mesenchyme — to the many clades which are required for the tooth attachment apparatus. Interestingly this cladogram is also viable from the evolutionary point of view, allowing the development of an ankylosis and its evolution into a gomphosis.

The tooth attachment found in an early mammal-like reptile, Thrinaxodon, appears in figures drawn by Crompton (1963) and Hopson (1964). Osborn studied these descriptions and figures, and concluded the teeth were probably ankylosed coronally and attached by fibrous tissue apically (Fig. 15). He suggested that this could be explained by considering that migration from the papilla into the follicle takes place during tooth development. The earliest migrating cells belong to clades of the more primitive reptilian tooth attachment type, and lead to the ankylosis coronally. Cells migrating later have differentiated further into clades which will produce the fibrous attachment (gomphosis), characteristic of mammals apically (Fig. 16).

Support for such a hypothesis comes from the work of Yoshikawa and Kollar (1981). In this study they dissected tooth germs from foetal mice of 16-19 days gestation and separated the tooth germ into three components — the dental sac (investing layer), papilla and enamel organ. The tissues were recombined in various combinations and after a short culture period were implanted in the eye. When enamel organ and dental sac were combined, in the 16 day material, 85% of the grafts produced a tooth, roots and supporting tissues, but this fell to only 15% with tissues from older embryos. In contrast, if enamel organ and papilla alone were recombined tooth, root and supporting tissues were found in 66% of the implants — regardless of embryo age (Fig. 17). These results indicate that, at least in the younger embryos, the dental sac and papilla have the same developmental potential, which is not surprising in view of their common origin from ectomesenchyme (Gaunt & Miles, 1967). But the fact that recombination of enamel organ and papilla usually resulted in tooth, root, cement, ligament and bone formation is strong evidence that migration from the papilla must have happened — at the very least regenerating the investing layer — supporting Osborn's hypothesis.

It is possible to find further evidence that cells of the dental papilla may be involved in periodontal development. If Hoffman's work (1960) is re-examined, he mentions two tooth germs which although surviving failed to grow and produce roots. Bone and periodontal ligament were formed however, and were found within the pulp. A later study, also by Hoffman (1966) demonstrated bone within the pulp of grafted teeth. This seems to indicate that progenitor cells for the periodontium exist within the pulp and in these cases failed to migrate out. So a further explanation of Hoffman's results (1960) is now possible. The adherent follicular cells may divide as Ten Cate suggests but the major source of cells is from the papilla, perhaps first renewing the investing layer which then produces the periodontium.

Also certain pathologies, such as radicular dentine dysplasia (Perl and Farman, 1977), lend weight to Osborn's hypothesis. In this condition rootless teeth develop and the pulp is filled with osteodentine resembling that found in certain fish and reptiles and an "ankylosis" type of attachment is present. Likewise in shell teeth (Rushton, 1954) intrapulpal collagen fibres attached to dentine are found. Recent work by Dick & Honoré (1984) in which intrapulpal periodontal tissue was found in odontomes from ovarian cysts, led them to propose that migration from the pulp to follicle of periodontal progenitor cells may take place.

Although this hypothesis may seem at odds with the current theory of periodontal development this is not the case. The vital role played by the investing layer is not disputed, this hypothesis suggests that the dental papilla can also contribute to periodontal development by migration of cells into the investing layer. It has already been mentioned that continuity between the papilla and investing layer exists, but many authors (Robins, 1967; Dahl, 1983) maintain that cell migration from the pulp cannot occur due to the presence of a pulp limiting membrane (Main, 1965). But descriptions of the pulp limiting membrane vary (Sicher, 1942a,b; Eccles, 1961) and its presence seems to be more apparent beneath the developing roots of erupting teeth (Main, 1965). Therefore it appears unlikely that the pulp limiting membrane, if it exists, presents any particular obstruction to cell migration.

1.5 Objectives of Study

The majority of investigations on cell movement in tooth development has been labelling studies using the rodent incisor (Chiba, 1965; Hwang and Tonna, 1965; Zajicek, 1974; Beertsen, 1975) and so are closely linked with the topic of eruption. The periodontal ligament has been studied by Gould et al (1980) who examined fibroblast migration in wound healing and by McCulloch and Melcher (1983) who examined cell movement within the healthy mature periodontal ligament.

Hoffman and Gillette (1964) in a labelling study describe centres of proliferative activity within the molar pulp and follicle during root development but made no attempt to trace cell movement. Ten Cate (1972) and Perera and Tonge (1981a) did describe cell movement within molar periodontal development but both studies were upon erupting teeth, cell movement presumably being linked with eruption.

The present study is unique, in that it is to investigate if cells migrating from the papilla might be the source of cells which produce the attachment apparatus. I am not aware of any previous study examining such a hypothesis or working on such early stages of molar tooth development.

2. MATERIALS AND METHODS

Tritiated thymidine when injected into animals, produces labelled DNA in those cells which were in the S-phase of mitosis at the time of injection. It is possible to visualize these cells and their progeny by means of autoradiography. By dividing the tissue under study into defined areas the change in labelling index (percentage labelled cells) with time can be used to determine if cells move from one region to another (Perera and Tonge, 1981a; McCulloch & Melcher, 1983).

2.1 Animals

Balb/c CR Chl (University of Alberta Animal Services) mice aged 10 and 13 days were injected with tritiated thymidine-methyl ^3H , specific activity 20 Ci/mmol (New England Nuclear, Boston, Mass.) at 1.0 μCi /gram body weight between 9-11 a.m. Mice from each age group were sacrificed by cervical dislocation at intervals of 4, 8, 12, 16, 24 and 48 hours after injection. In all age groups at least five mice for each time interval were used. Unlabelled mice of the same ages were used as controls, to provide a reference of the emulsion's background grain count.

2.2 Histology

Immediately after death the mandibles were dissected from the jaws and fixed in neutral buffered formalin for at least 48 hours. They were then decalcified for 4-5 days in formic acid — complete decalcification being verified by taking radiographs of randomly selected specimens. After dehydration in ascending concentrations of ethanol and routine processing the mandibles were embedded in paraffin wax. Serial sections were cut bucco-lingually at 5 μm thickness and placed on gelatin coated slides.

The tooth chosen for this study was the mandibular third molar for the following reasons:

1. It develops after birth (Hay, 1961; Cohn, 1957; Gaunt, 1964, 1966) and so it is possible to

select more accurately the stage of development at which the label is given (any runts or giants within litters were not used).

2. It has a simple shape.
3. Only a single root develops.
4. It is the smallest tooth in the molar series and therefore cell counts are less time-consuming.

2.3 Autoradiography

Both experimental and control (unlabelled) slides were coated with Ilford L4 emulsion (Ilford Ltd, England) diluted 1:1 with distilled water, according to the liquid emulsion dipping technique of Rogers (1967). After drying the slides were placed in light-proof boxes and stored at 4°C. After 4 weeks exposure the slides were developed in Dektol developer (Eastman Kodak, Rochester, N.Y.) at 17-18°C for 5 minutes, rinsed in running tap water and fixed in Kodak Rapid Fix at 17-18°C for 5 minutes then finally washed in running tap water for 5 minutes. The slides were then lightly stained with haematoxylin and eosin through the emulsion.

2.4 Analysis

For each specimen three sections from the centre of the tooth germ, separated from each other by at least 15 μm were chosen for analysis. Torn or folded sections were discarded and adjacent undamaged sections used.

Photomicrographs of the tooth germ and surrounding tissue were taken at a magnification of 250x using Kodalith film (Kodak Canada Inc., Toronto, Ont.) and printed on Ilford Multigrade photographic paper to produce a total magnification of approximately 550x. Usually 2-3 photomicrographs were required for each section and therefore a montage of each was prepared.

Outlines of the tooth germ and bone were traced using tracing film and the positions of all labelled and unlabelled cells in certain defined areas were marked. The following method of

defining areas was used (Fig. 18).

1. A line was drawn vertically, bisecting the tooth germ and using midpoints between cervical loops (the apical rim of the enamel organ) and cusp tips as guides.
2. A line was drawn joining the left and right cervical loops following the obvious change in cell density between dental papilla and follicle.
3. Lines at right angles to the vertical bisecting line were drawn at distances of 50 μm and 100 μm into the pulp from the line across the base of the pulp.
4. Lines from the tip of the cervical loop to bone, at right angles to a tangent from the curvature of the loop were drawn.
5. Finally lines from the outer enamel epithelium to bone, 50 μm coronal to the cervical loop, at right angles to a tangent from the curvature of the outer enamel epithelium were drawn.

These lines divided the tooth germ and surrounding tissue in half, each half having four areas — two within the papilla and two in the surrounding follicle.

In addition a further area (PL) was defined — a 10 μm wide band across the base of the pulp was drawn (Fig. 19).

After marking the labelled and unlabelled cells on the tracing a check was made that no cells had been missed by reference to the slides using magnifications of 400x and 1000x. Endothelial cells were not marked and were distinguished by their shape, location and their nuclei bulging into the blood vessel lumen. Within the pulp odontoblasts and preodontoblasts were also excluded from marking.

A cell was considered to be labelled if 5 or more silver grains overlay its nucleus. All cell counts and later calculations were performed on independently marked material — at no time was the age group or time since injection known to the investigator.

2.5 Labelling Index

The labelling index (L.I.) for each area was plotted against time. The L.I. being defined as:

$$\text{L.I.} = \frac{\text{Number of labelled cells}}{\text{Total number of cells}} \times 100$$

By reference to previous studies (Gould et al, 1980; Perera and Tonge, 1981a; McCulloch and Melcher, 1983) it should be possible to predict how cell movement between areas would appear graphically.

1. No movement — The graph would be similar for each area, showing similar rises and falls in the L.I. at approximately the same times in every area.
2. Movement between areas — There would be a lack of synchrony between graphs. Areas gaining cells would show a rise in L.I. and those reciprocally losing cells would show a fall at about the same time.

In this way it should be possible to follow cell movement between areas if it exists and hence if cells migrate from papilla to follicle.

3. RESULTS

All data were expressed as the mean labelling index ($\pm$ Standard Error) and are presented as graphs. Student's t test was used to determine if any significant difference between points existed. No significant difference between right and left teeth or between buccal and lingual was found and therefore the results were pooled.

3.1 Control

Slides prepared from unlabelled mice were found to have the same low background grain count as slides which had been prepared from mice which had received injections of tritiated thymidine. In general grain counts over individual labelled cells was impossible as such high numbers of silver grains were deposited over the labelled cell (Plates 1 and 2).

3.2 10 Day Old Group

3.2.1 Area 1

The mean L.I. curve against time for area 1 is shown in Figure 20A. At 4 hours the L.I. was $9.27 \pm 0.86\%$, rising to $11.41 \pm 0.75\%$ at 8 hours then falling to $9.79 \pm 1.13\%$ at 12 hours. Thereafter it reached a plateau for the remainder of the experiment, being $10.69 \pm 0.54\%$ after 48 hours (Tables 1 & 2).

3.2.2 Area 2

In this area the L.I. rose from $6.02 \pm 0.62\%$ at 4 hours to $8.54 \pm 0.44\%$ at 8 hours, which was found to be significant ($p < 0.05$). This was followed by a significant fall to $5.93 \pm 0.82\%$ at 16 hours ($p < 0.05$) and a subsequent rise to $8.12 \pm 0.93\%$ at 24 hours. The L.I. after 48 hours was $8.09 \pm 1.00\%$ which was not appreciably different (Fig. 20B, Tables 1 & 2).

3.2.3 Area 3

At 4 hours the L.I. was $3.26 \pm 0.40\%$ and remained at about this level, being $3.05 \pm 0.46\%$ at 12 hours then rising to $4.48 \pm 0.83\%$ at 16 hours. From 16 hours onwards the L.I. reduced slightly reaching $3.11 \pm 0.52\%$ at 48 hours (Fig. 20C, Tables 1 & 2).

3.2.4 Area 4

In this area the L.I. reduced from $11.32 \pm 0.44\%$ at 4 hours to $8.76 \pm 1.10\%$ at 12 hours. There followed a rapid and significant ($p < 0.05$) rise to $12.43 \pm 1.05\%$ at 16 hours. The L.I. then remained essentially unchanged, finishing at $11.89 \pm 0.82\%$ after 48 hours (Fig. 20D, Tables 1 & 2).

3.2.5 Area 3 & 4

A mean L.I. was calculated for area 3 & 4 combined (i.e. pulpal areas) in order to make later interpretation of the graphs easier. At 4 hours the mean L.I. was $7.71 \pm 0.35\%$ which dropped significantly ($p < 0.05$) to $6.06 \pm 0.48\%$ after 12 hours. A significant rise ($p < 0.01$) to $8.60 \pm 0.46\%$ at 16 hours followed and then the L.I. remained at approximately this level, reaching $8.01 \pm 0.66\%$ after 48 hours (Fig. 20E, Tables 1 & 2).

3.2.6 Across the Base of the Papilla — Area PL

An examination of the L.I. in the $10 \mu\text{m}$ band stretching across the base of the tooth germ in the 10 day old group showed a rise to a maximum of $6.09 \pm 1.05\%$ in the first 12 hours after which it reached a plateau (Fig. 20F, Tables 1 & 2).

3.3 13 Day Old Group

3.3.1 Area 1

The L.I. climbed rapidly to peak at $10.31 \pm 1.42\%$ at 8 hours after which it declined, with a significant ($p < 0.05$) reduction between 16 and 24 hours to a level of $5.51 \pm 1.20\%$ at 24 hours. It then remained stable at this level, finishing at $5.76 \pm 0.91\%$ at 48 hours (Fig. 21A, Tables 3 & 4).

3.3.2 Area 2

At 4 hours the L.I. had reached $6.28 \pm 1.07\%$ but then fell to $5.21 \pm 1.02\%$ at 8 hours. It then climbed in the next eight hours to reach $7.45 \pm 0.64\%$ at 16 hours. A significant ($p < 0.05$) drop to $4.44 \pm 0.91\%$ at 24 hours was followed by a plateau with the L.I. being $4.54 \pm 0.96\%$ after 48 hours (Fig. 21B, Tables 3 & 4).

3.3.3 Area 3

The L.I. in this area reached $2.95 \pm 0.34\%$ at 4 hours and remained stable, being $2.60 \pm 0.59\%$ at 8 hours. There was then a definite rise and fall in the L.I., a significant ($p < 0.05$) rise from 8 hours to reach $4.57 \pm 0.40\%$ at 12 hours and then a significant fall ($p < 0.01$) from $4.85 \pm 0.47\%$ at 16 hours to $2.61 \pm 0.55\%$ at 24 hours. A gradual decline to $3.46 \pm 0.29\%$ after 48 hours followed (Fig. 21C, Tables 3 & 4).

3.3.4 Area 4

The shape of the graph resembled that obtained from area 3. The L.I. reaching $10.62 \pm 1.56\%$ after 4 hours, dropping slightly to $10.08 \pm 1.56\%$ at 8 hours and then rising to a peak at 16 hours of $12.17 \pm 0.79\%$. A significant drop ($p < 0.05$) to $8.71 \pm 1.22\%$ after 24 hours was followed by a gradual fall to $7.44 \pm 0.77\%$ by 48 hours (Fig. 21D, Tables 3 & 4).

3.3.5 Area 3 & 4

The L.I. reached $7.09 \pm 0.99\%$ by 4 hours and peaked at $8.77 \pm 0.53\%$ after 16 hours. A significant drop ($p < 0.01$) to $5.92 \pm 0.80\%$ at 24 hours then took place thereafter remaining essentially steady, being $5.55 \pm 0.45\%$ by 48 hours (Fig. 21E, Tables 3 & 4).

3.3.6 Across Base of the Papilla — Area PL

In general the L.I. in the 13 day old group was significantly lower ($p < 0.05$) than that of the 10 day old group. At 4 hours the L.I. was $2.64 \pm 0.77\%$ then fell to $1.58 \pm 0.67\%$ at 8 hours before rising to $3.31 \pm 0.54\%$ by 12 hours. It remained steady until 16 hours and then fell significantly ($p < 0.05$) to $1.14 \pm 0.43\%$ at 24 hours before rising to $2.10 \pm 0.55\%$ at 48 hours (Fig. 21F, Tables 3 & 4).

3.4 Comparison of 10 Day And 13 Day Old Groups

The shape of the graphs (Fig. 22A) in area 1 was similar until 16 hours. At this time there was a significant fall ($p < 0.05$) in the 13 day old group until 24 hours after which it remained at a plateau. In contrast the 10 day old group remained at a steady level essentially from 12 hours onwards. There was a significant difference between the two age groups from 24 ($p < 0.05$) to 48 hours ($p < 0.001$) (Table 5).

Differences between the results obtained for area 2 (Fig. 22B, Table 5) are interesting. In the 10 day old group there was a rise in L.I. peaking at 8 hours then falling to its minimum level by 16 hours. In contrast the older, 13 day, material fell from a peak at 4 hours to a minimum at 8 hours and then rose to peak again at 16 hours, thus giving an almost mirror image of the 10 day material. The difference between the peak at 8 hours in the 10 day old group and minimum at 8 hours in the 13 day old group was significant ($p < 0.01$). From 16 hours to 24 hours the graphs also differed, in the 10 day old group the L.I. rose and in the 13 day old group it fell significantly ($p < 0.05$). Thereafter both graphs maintained a plateau, with the 10 day old group being at a significantly higher level than the 13 day group ($p < 0.01$ at 48 hours).

In area 3 the graphs were similar (Fig. 22C, Table 5) until 8 hours and then from 8 to 12 hours the 13 day L.I. showed a significant rise ($p<0.05$) whilst the 10 day L.I. remained steady. At 12 hours the 13 day L.I. was significantly higher ($p<0.05$) than the 10 day L.I. Both 10 and 13 day L.I. then increased between 12 and 16 hours and then fell slowly in the 10 day old group during the remainder of the experiment. The 13 day old group also fell significantly ($p<0.01$) between 16 and 24 hours and then showed a slight rise over the remaining 24 hours.

Marked differences were seen between graphs of 10 and 13 day labelling indices in area 4 (Fig. 22D, Table 5). Up to 8 hours the graphs were similar but between 8 and 16 hours the 10 day old group showed a marked fall then rise whilst the 13 day old group showed a steady rise over this time period. After 16 hours the 10 day old group reached a plateau whereas a significant fall ($p<0.05$) in L.I. in the 13 day old group between 16 and 24 hours took place followed by a gradual fall over the remaining 24 hours. The L.I. at 48 hours was significantly higher ($p<0.01$) in the 10 day old group.

If areas 3 and 4 are combined and then compared between age groups the graphs were similar (Fig. 22E, Table 5) until 8 hours after which the 10 day old group fell to a minimum at 12 hours and then increased significantly ($p<0.01$) to 16 hours. In contrast the 13 day old group showed a steady rise over the same time period. From 16 hours onwards the 10 day old group remained at a plateau whilst the 13 day old group fell significantly ($p<0.01$) and then also reached a plateau — but at a significantly lower level ($p<0.01$) than the 10 day old group.

Large differences were seen if the L.I. of area PL (the band across the base of the papilla) were compared (Fig. 22F, Table 5). The L.I. in the 10 day old group was almost always significantly higher ($p<0.05$) than the 13 day old group. The L.I. of the 10 day old group climbed rapidly, reaching a maximum at 12-16 hours and then remained almost unchanged. In contrast the L.I. of the 13 day old group showed a fall between 4 and 8 hours followed by a rise until 12 hours whereupon it remained steady but fell between 16 and 24 hours. From 24-48 hours the L.I. then increased slightly.

3.5 Comparison of Areas

If the graphs of areas 2, 3, 4 and the band across the base of the pulp (PL) are compared (Fig. 23A) it can be seen that the shapes were almost identical in the 13 day old group. All rose to a peak at 4 hours then dropped by 8 hours before steadily rising to a maximum at 16 hours. A significant fall ($p < 0.05$) in all areas between 16 and 24 hours was then followed by a plateau. However, area 1 was somewhat different, it rose to a maximum at 8 hours and then fell steadily to 24 hours after which it levelled off.

In contrast the graphs of L.I. obtained from the 10 day old group showed a marked variation from area to area and none followed the pattern exhibited by the 13 day areas 2, 3, 3 & 4 and PL (Fig. 23B).

3.6 Description of Proliferative Activity

The 10 day old material was found to be in the late bell stage of development with morphogenesis well advanced (Plate 3). Labelling was found in cells dispersed throughout the papilla but was particularly heavy in a broad zone lying beneath the odontoblasts and a centre of proliferation was invariably seen in an area lying approximately 70 μm from the cervical loop within the pulp and adjacent to the odontoblasts (Fig. 24A). Labelled cells were present in central parts of the papilla and at the base, but tended to be few in number. Labelled cells were often seen at the boundary between papilla and follicle. Within the follicle (areas 1 and 2) labelled cells were also present — and tended to be more frequent in area 1 (Fig. 24A).

By 13 days the tooth germ had laid down a considerable thickness of dentine and enamel (Plate 4). The definitive outline of the crown was being developed and 48 hours later was nearly complete. Labelled cells were fewer but the proliferative centre above the cervical loop region was still active and more labelling in area 1 than area 2 was seen (Fig. 24B).

4. DISCUSSION

4.1 Rationale of Technique Adopted

The labelling and autoradiographic technique chosen, which measures the change in labelling index with time to determine if cells move between regions is well established and has been used many times in studies of tooth eruption and the periodontal ligament (Zajicek, 1974; Beersten, 1975; Perera and Tonge, 1981a; McCulloch and Melcher, 1983).

In common with a large number of previous studies the present study used tritiated thymidine to label the cells. Tritiated thymidine is almost exclusively incorporated into DNA during the S-phase of the mitotic cycle and, apart from a small proportion of mitochondrial DNA, remains within the nucleus throughout the life of the cell (Amano et al, 1959; Baserga and Kisielski, 1963; Cleaver, 1967; Feinendegen, 1967; Baserga and Malamud, 1969). It is possible for tritiated thymidine to be reutilized after cell death (Cleaver, 1967) but this does not seem to be relevant in the present study as a review of the dental literature gives no mention of cell death taking place in normal tooth and periodontal development. However, the β -particles which result from the decay of the tritium may result in toxic effects on the labelled cells, such as changes in the mitotic rate or even cell death (Feinendegen, 1967) but these effects are considered to be small in whole animal experiments which use 1-2 μ Ci/g body weight of tritiated thymidine (Cronkite, 1959; Smith et al, 1962; Feinendegen, 1967). In addition if any tritium were salvaged from dead cells and reutilized during 48 hours of tooth and periodontal development it seems most unlikely that results would be affected since Stocker and Pfeifer (1967) showed a level of only 3-4% tritiated thymidine due to reutilization compared to the levels of circulating label produced by the initial pulse.

Of more importance in this study is the time after injection during which circulating label is available to cells. This has been shown to be less than 1 hour (Hughes et al, 1958; Rubini et al, 1960; Staroscik et al, 1964; Chang and Looney, 1965; Cleaver, 1967). In view of the short duration of the availability of label the pulse-chase method (using a labelled "pulse" followed

by an unlabelled "chase" of thymidine) used in systems which potentially have a pool of label present for a long time - such as *in vitro* cultures or in chick embryogenesis (Sauer and Walker, 1961; Cleaver, 1967) - was not used. In addition due to the small size and young age of the mice used in this study it does not seem appropriate to subject them to the added trauma of a chase injection of unlabelled thymidine.

4.2 Analysis of Results

At first sight the results for the 10 day material (Fig. 20 A-F) appear very confusing. To simplify matters it is easier to discuss the results from the 13 day old group first.

4.2.1 13 Day Old Group

When the shapes of the graphs of areas 2, 3, 4 and PL are compared (Fig. 25A) they can be seen to be very alike. This would seem to indicate that there is no movement of cells between these areas. It is reasonable to assume that the similar changes in the shape of each graph with time could be the result of changes in the labelling index due to the mitotic cycle (Fig. 26). The cycle is divided into four phases — G_1 , S, G_2 and M (Alberts et.al, 1983). A cell actually divides during phase M which usually lasts about an hour and each daughter cell now enters period G_1 (=Gap 1) which is of variable length. This is followed by the S or synthetic phase, during which the DNA is copied resulting in a tetraploid cell. The cells now enter G_2 during which the materials necessary for mitosis are accumulated and the cell now divides again. Daughter cells now may re-enter the same cycle or may enter a quiescent state (G_0) from which they may differentiate or be induced to re-enter G_1 .

Bearing this in mind, all 13 day graphs except area 1 (Fig. 23A) show a peak at 4 hours and another at 12-16 hours, with a fall in labelling index between these times. This may be caused by any of the following reasons:

1. The labelling index peaks at 4 hours as the labelled cells have already undergone their first division. This is followed by a fall as the surrounding unlabelled cells divide. The second

peak results from a second mitosis of labelled cells. This would imply a cell cycle of about 12 hours which is rather short, but has been suggested by Osman and Ruch (1978).

2. At 4 hours none of the labelled cells has yet divided. The fall from this time to 8 hours is caused by cells which were in G_2 at the time of injection (and therefore not becoming labelled) dividing. The subsequent rise is the result of the labelled cells now dividing. This would imply a rather extended G_2 and a much longer cell cycle, in the order of 20-30 hours, which is more in keeping with values derived in other studies (Perera and Tonge, 1981b; Dahl, 1983).
3. In no area is the fall in L.I. between 4 and 8 hours significant so perhaps little importance should be attached to it. Indeed Perera and Tonge (1981a) and McCulloch and Melcher (1983) do not consider the possibility of the mitotic cycle influencing their results.

However, the fact that a similar rise, fall and rise in L.I. occurs in 4 different areas [2, 3, 4 and PL (Fig. 25A)] suggests that it is not merely due to a repeated experimental error.

Although it is interesting to speculate on the possible causes of the characteristic graph shapes of these areas the fact remains that no interpretation suggests that cells are moving in this age group. In addition these results indicate that cells of the papilla and the base of follicle probably have a similar generation time, although the proportion of dividing cells in each region is different.

The striking difference between the shape of the graph for area 1 (Fig. 23A) and all the other areas - where area 1 lacks the characteristic drop in L.I. at 8 hours - suggests that it behaves in a different way. This is probably the case but will be considered when the 10 day old group is discussed.

4.2.2 10 Day Old Group

In contrast to the 13 day old group the graphs of L.I. with time in areas 2, 3, 4 and PL for the 10 day old mice are quite different from each other (Fig. 25B). Analysis of these graphs and their differences is difficult, but is made easier if the following points — which may be

assumed from the 13 day results — are applied.

1. The cell generation time of cells in the papilla and follicle are about the same. Therefore differences in graph shape in the 10 day old mice are probably not the result of radically different mitotic rates.
2. The graph shapes obtained from the 13 day old group can be used as a baseline; i.e. similar shaped graphs would have been observed for the 10 day mice if cells and their daughters had remained within the same region during the 48 hours of the experiment.

If these points are valid it means that the variation in L.I. between areas in the 10 day mice is probably the result of cell movement between regions. In order to determine the direction of this movement it is simplest to compare the graph shapes of areas 2 and 3 & 4 first (Fig. 20B,E). It seems that cells must be entering area 2 (the follicle) because its labelling index rises until 8 hours and the concomitant drop in labelling index in area 3 & 4 (the papilla) from 4-12 hours suggests that this is from where the cells have come. Also within area 2 of the 13 day mice the labelling index is reduced at 8 hours — the opposite of what is found in area 2 of the 10 day mice (Fig. 22B). In addition the labelling index of area 3 & 4 in 10 day old mice should have been rising by 12 hours, not be at its minimum value (Fig. 22E).

Further support is provided by comparing the graphs of area PL for 10 and 13 day animals (Fig. 22F). If as suggested here, cells move from the papilla to the follicle during day 10 they must pass through area PL and therefore a difference in graph shape from that in day 13 should result. This can be seen to be the case, with the 13 day graph of area PL following the same shape as the other areas. But in the 10 day animals the shape is quite different with the labelling index rising steadily to a plateau (Fig.22F). This may be interpreted as being caused by a movement of cells through area PL, which acts rather like a corridor between two rooms - areas 3 and 2, and also causes cell movement through the area to be slowed (Fig. 27A,B). There is a steady rise as the first of the labelled cells enter the area and eventually a steady state is reached where the number of labelled cells entering and leaving the area is approximately equal (Fig. 20F). It is unlikely that the increase in the L.I. of area PL is the result of proliferation of

cells within area PL as no groups and few couplets of labelled cells were seen and the low level of the labelling index of area PL in the 13 day mice implies it is not a proliferative area.

If the idea of cell movement from papilla to follicle is correct it should be supported by the results obtained from areas 3 and 4 if they are examined separately — and this is found to be so. Descriptions of the proliferative area within the papilla (Fig. 24A) and the difference in labelling indices place it largely within area 4, therefore area 3 should, to some extent, also act as a "corridor".

An indication that this is so is provided by the steady labelling index in area 3 between 4 and 12 hours instead of the expected fall (Fig. 22C) — implying a net gain of labelled cells. During the same time period there is a decrease in labelling index in area 4 instead of the expected rise between 8 and 12 hours (Fig. 22D). So it appears that cells are moving from area 4 to area 3. Other explanations for such a drop in area 4 would involve consideration of the mitotic cycle (for instance unlabelled cells dividing). However, if this were so the graph shape for area 3 would duplicate that of area 4. There is probably a loss of cells from area 4 in a coronal direction caused by growth of the crown in height, but the graphs of areas 3 and PL indicate a cervical movement as well.

A common finding on all graphs is the plateau in the labelling index between 24 and 48 hours (Figs. 20A-E and 21A-E). This may be the result of a steady state system being arrived at by 24 hours.

Having said this an anomaly appears to exist with regard to area 1 in both age groups (Fig. 22A). Their graphs representing changes in L.I. with time have totally different shapes when compared to other areas. An insight into the cause may be gained from the work of Hoffman and Gillette (1964) and Ten Cate (1972) who in labelling studies on more advanced stages of tooth development noted a proliferative area within the follicle lateral to the cervical loop (i.e. in area 1) and this was also noted in the present study (Fig. 24A,B). Studies by Ten Cate (1972) and Perera and Tonge (1981a) showed a movement of cells from this area coronally. The shape of the graph of area 1 from the 13 day mice (Fig. 21A) is exactly what

could be expected from such a proliferative area which is allowing cells to move away coronally and which is independent of other areas, the rise in L.I. to a peak at 8 hours being caused by division of the labelled cells within this area, the subsequent fall, [not seen in the 10 day group (Fig. 22A).] may be the result of a movement of labelled cells out of the area.

Area 1 in the 10 day old mice is a different story. The graph remains identical with that of area 1 in the 13 day mice until 12 hours and then instead of falling it remains stable (Fig. 22A). It is proposed that area 1 in the 10 day old mice also acts as a proliferative centre but unlike the condition in 13 day old mice it is not independent. The expected fall between 16 and 24 hours does not take place because cells move into area 1 from area 2. Again after 24 hours a steady state exists. But at some time between 10 and 13 days the movement of cells into area 1 from area 2 ceases (or at least is not detectable by the method used in this study.)

A study of the graphs reveals that movement in the reverse direction (i.e. from follicle to papilla) does not seem possible. For example areas 1 and 2 peak at the same time in the 10 day mice (8 hours) and the L.I. is falling between 8 and 12 hours in area 4 which is the time during which it should be gaining cells if they were migrating into the papilla. In addition, such a movement would suggest that cells move apically from the coronal region - the opposite to the direction found during eruption a short time later (Ten Cate, 1972; Perera and Tonge, 1981a).

4.3 Proposed Theory of Periodontal Development

In view of these results and the study by Yoshikawa and Kollar (1981) it is reasonable to accept Osborn's (1984) hypothesis that cells migrate from the papilla to the follicle. An important feature of the present study is that it has shown that such a migration takes place in normal tooth and periodontal development whilst most of the earlier work was performed upon tooth germs which had been subjected to surgical procedures (Hoffman, 1960; Ten Cate et al, 1971; Freeman et al, 1975). Even the work of Yoshikawa and Kollar (1981) is open to such criticism. It is possible that such surgery followed by growth in "foreign" areas results in abnormal development. Alternatively the transplanted components of the tooth germ may be

able to regulate their development and the results obtained may then only be valid for artificial conditions. But in view of this present study this does not seem to be the case.

Although the results of this study seemingly contradict the current idea that the investing layer of the follicle is the source of the periodontium (Ten Cate, 1969) this is not so. Instead it is proposed that late during crown development the cells of the papilla act as a source of cells for the follicle. An important point to note is that in the transplantation studies of Ten Cate et al (1971) not only the investing layer but also the enamel organ and papilla cells were labelled. So strictly speaking the results of these studies can only be interpreted as an indication that the periodontal tissues are derived from donor material — it cannot be concluded that only the investing layer was involved. In addition the work cited by Ten Cate in support of the idea that the investing layer is the source of the periodontium [for example Hoffman (1960), Ten Cate et al (1971), Freeman et al (1975)] may also be explained by cells migrating from the papilla to the follicle.

The dental pathology, radicular dentine dysplasia (Perl and Farman, 1977), mentioned in the introduction, may be explained by a failure of movement of cells from the papilla to the follicle. The ankylosis attachment found in these teeth is interesting — lending weight to Osborn's hypothesis regarding the development of the tooth attachment found in Thrinaxodon. If Osborn is correct, it is possible in the case of radicular dentine dysplasia, that migration of cells from the papilla takes place only for a short time. Therefore only the clades of cells responsible earlier in evolution for producing the reptilian form of attachment (the ankylosis) have moved before migration ceases.

The intrapulpal periodontal tissues observed by Hoffman (1960) and Dick and Honoré (1984) may be considered to have resulted from the differentiation of periodontal progenitor cells which have failed to migrate. These observations cannot be explained by Ten Cate's (1969) theory of periodontal development from the investing layer.

It seems likely that the periodontium is derived from both the papilla and the investing layer of the follicle. This study has examined an earlier stage of periodontal development — with

the papilla acting as an additional source of cells for the follicle which later produces the periodontium - although it is possible that the investing layer is entirely derived from the papilla early in development. But after a certain stage of development (about 12-13 days for M₃ in the mouse) the follicle probably becomes self-sufficient and the movement of cells from the papilla stops. The self-sufficiency of the mature periodontal ligament seems clear as a long-standing clinical impression is that the periodontal condition of root-filled teeth remains good in the presence of adequate oral hygiene (Miller, 1962).

4.4 Future Work In This Field

Additional evidence supporting the theory of migration of cells from papilla to follicle will probably be required. A relatively straightforward study to show this would be to repeat Yoshikawa and Kollar's study (1981), only to label each component of the tooth germ whilst they are being separately cultured *in vitro*. By recombining labelled and unlabelled components in different combinations the fate of the papilla cells should become clear.

Similar studies could be designed but using cells of different H-2 histocompatibilities and transplanted into an F1 hybrid. The fates of different cell populations could then be determined by the use of specific α -H2 antibodies.

4.5 Summary

This work has provided further evidence in support of Osborn's (1984) hypothesis, that cells move from papilla to follicle and play a role in periodontal development. However, beyond a certain stage in development the follicle becomes independent of the papilla.

As discussed by Yoshikawa and Kollar (1981), the ability of the dental papilla to contribute to periodontal development raises important issues regarding tooth transplantation and periodontal regeneration.

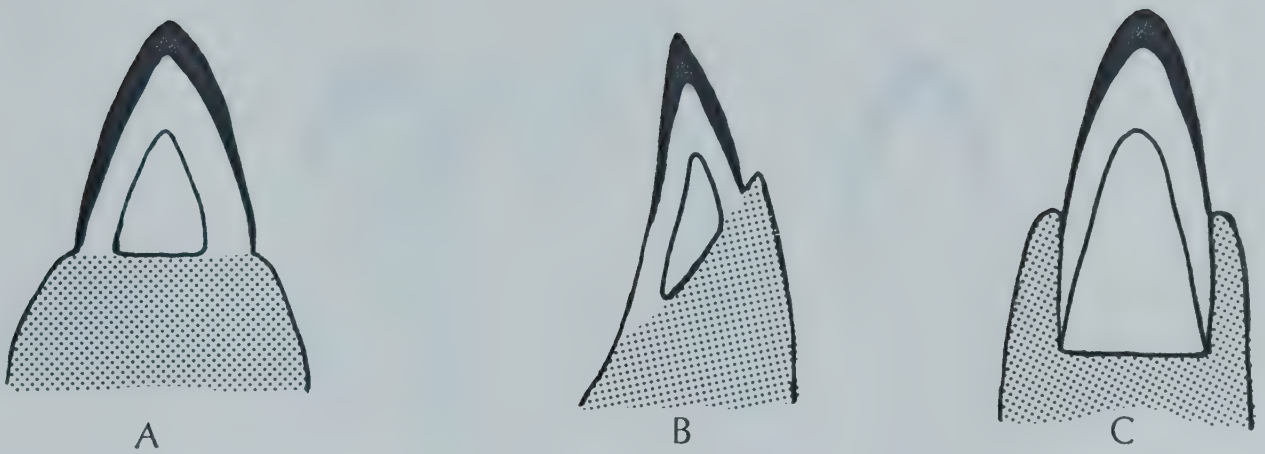


Fig. 1. Forms of ankyloses A. Acrodont ankylosis. B. Pleurodont ankylosis. C. Thecodont ankylosis.

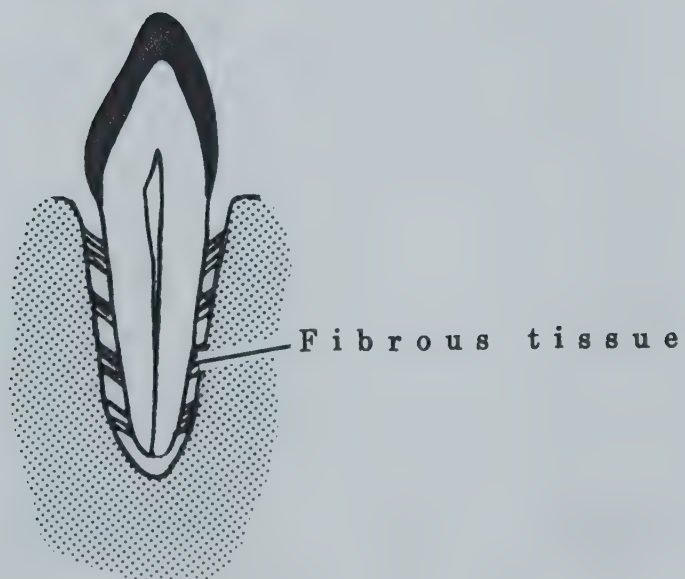


Fig. 2. Diagram of a mammalian gomphosis.



A . R e p t i l i a n



B . M a m m a l i a n

Fig. 3. Diagram of A. Reptilian tooth B. Mammalian tooth, showing lengthening, tapering and closure of apex of mammalian tooth.

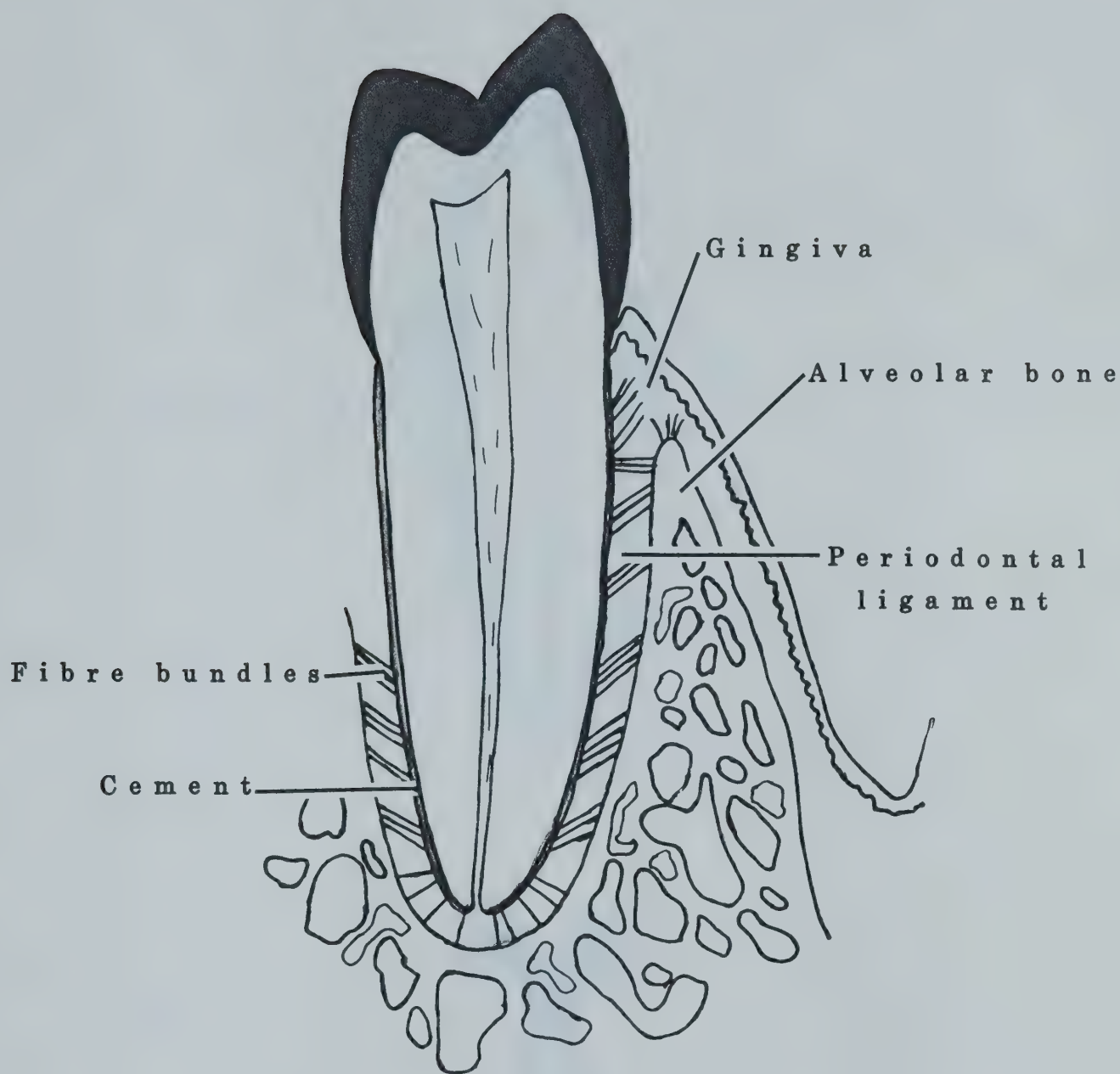


Fig. 4. The normal periodontium.

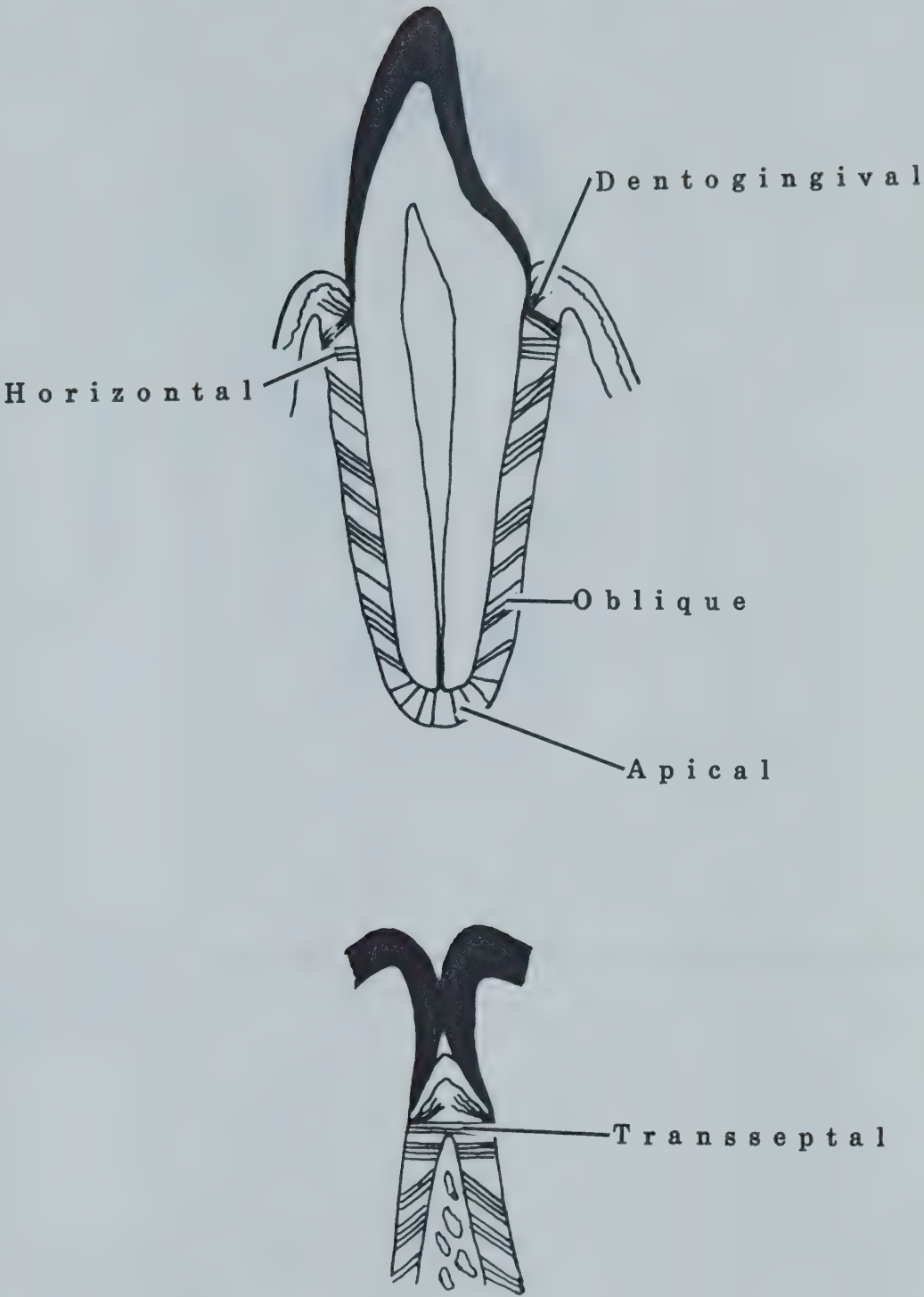


Fig. 5. The orientation of some principal fibre bundles in the periodontal ligament.

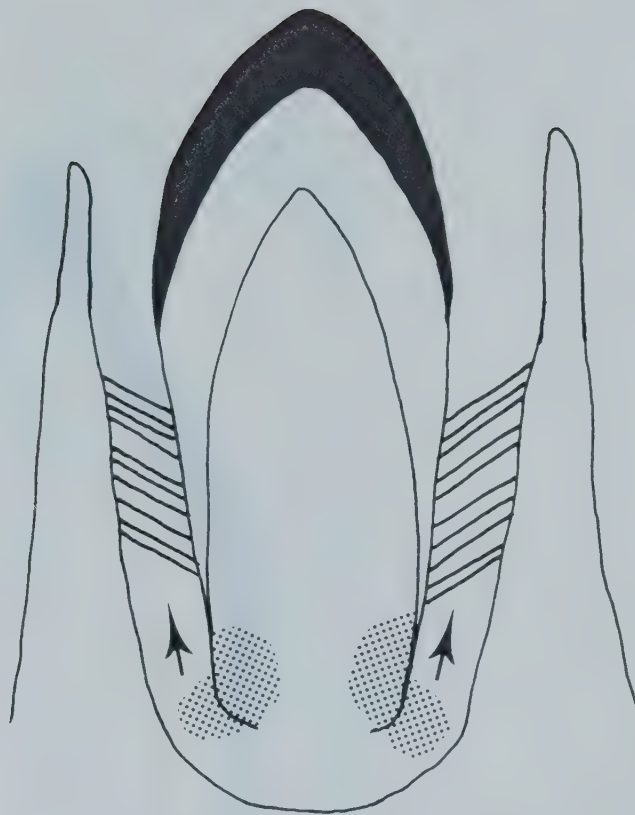


Fig. 6. Sites of cell division within the periodontal ligament. (Arrows indicate direction of cell movement.)

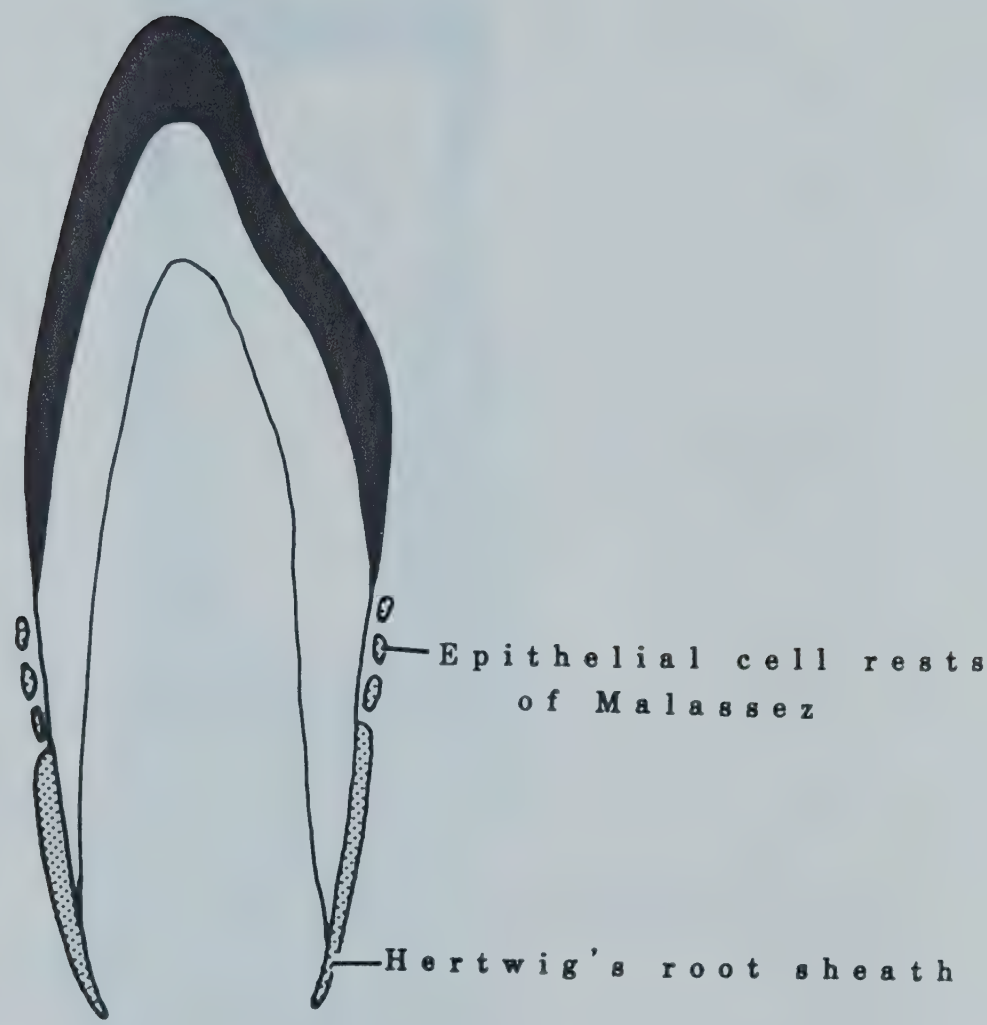


Fig. 7. Diagram of a developing tooth root, showing Hertwigs root sheath and the Epithelial cell rests of Malassez.

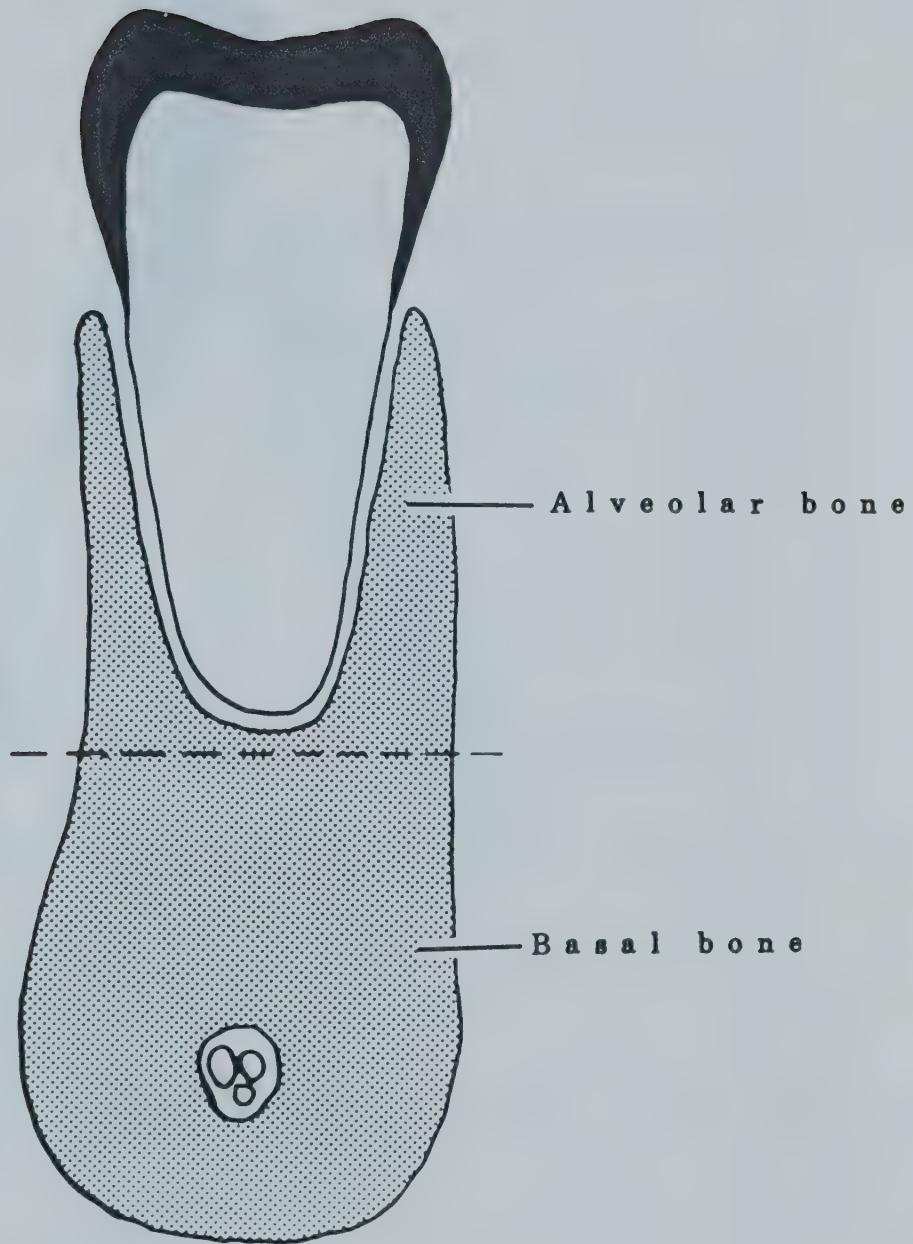


Fig. 8. The arbitrary division between alveolar and basal bone.

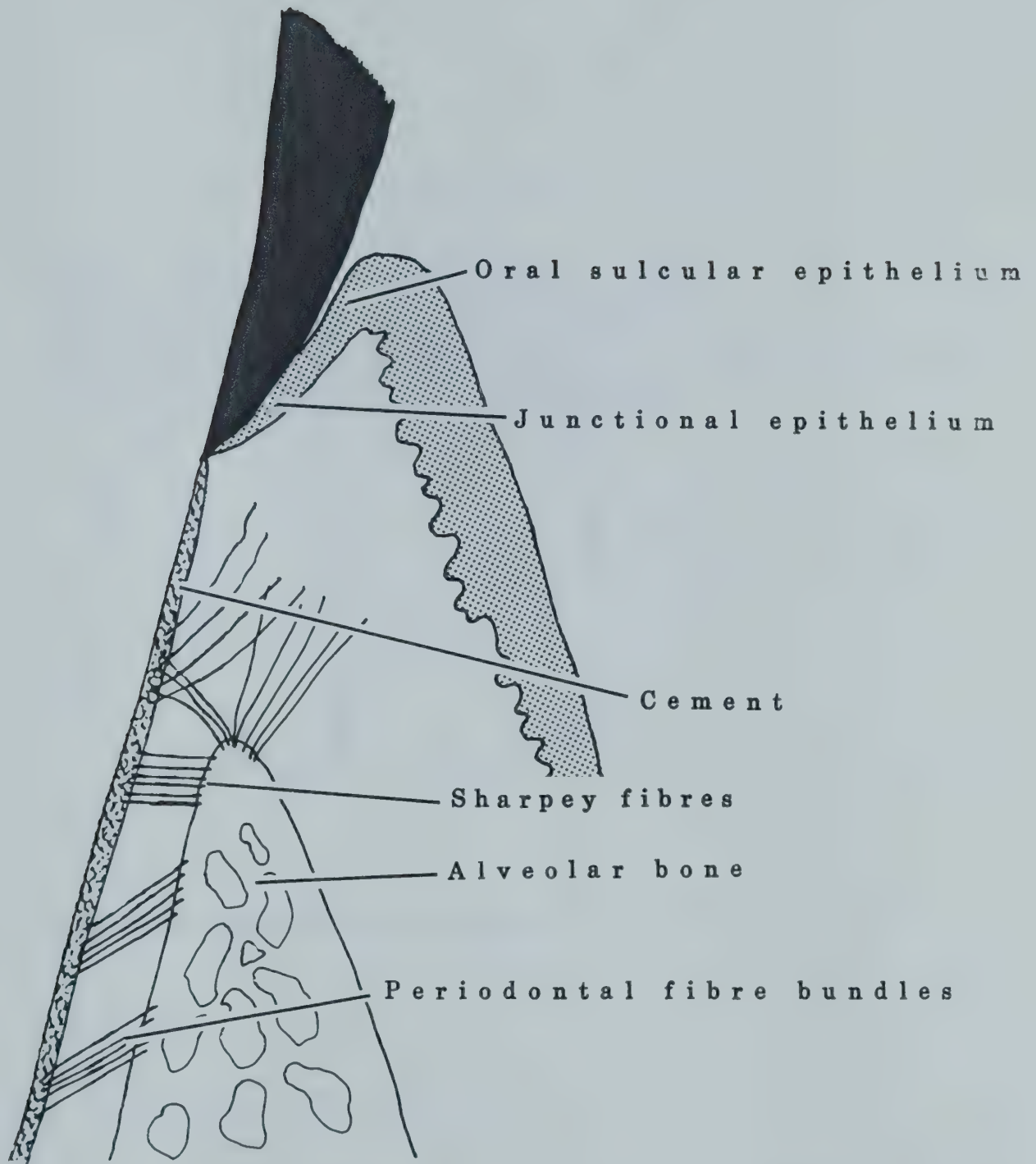


Fig. 9. Diagram of a section of a tooth showing the gingiva — junctional epithelium and oral sulcular epithelium.

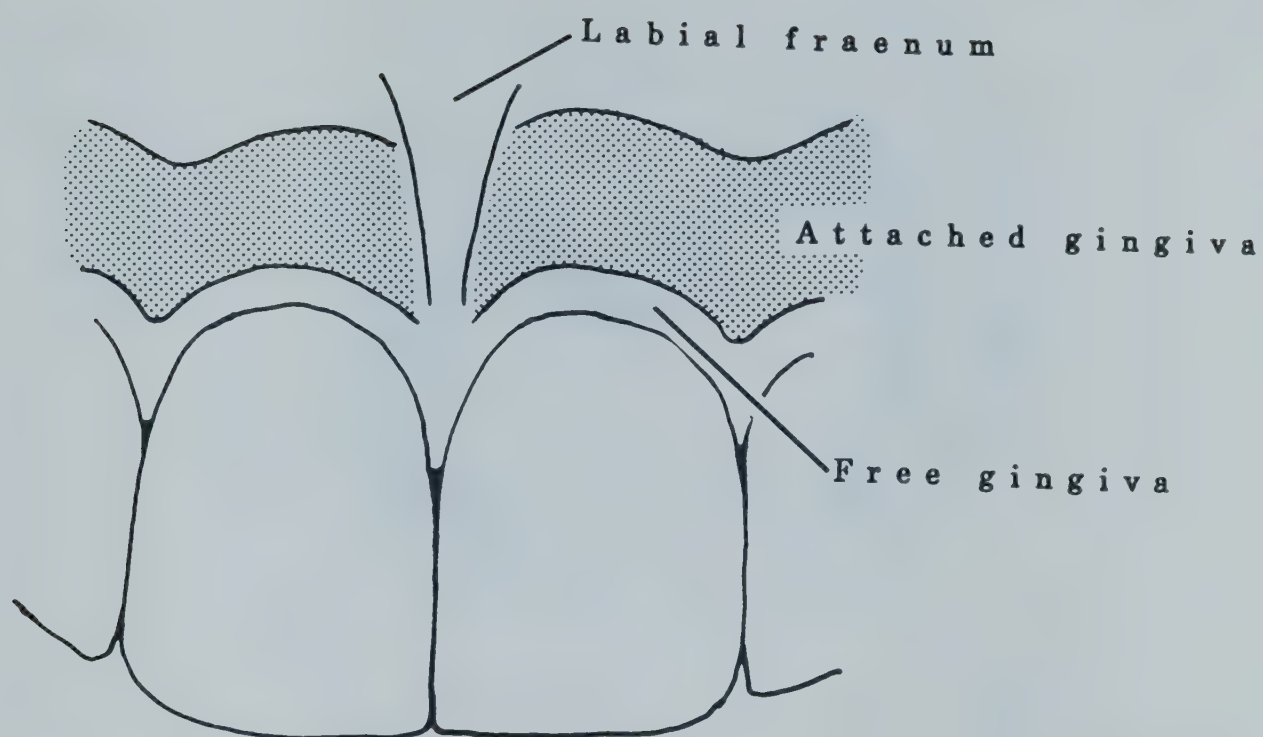


Fig. 10. Appearance of the labial surface of the gingiva.

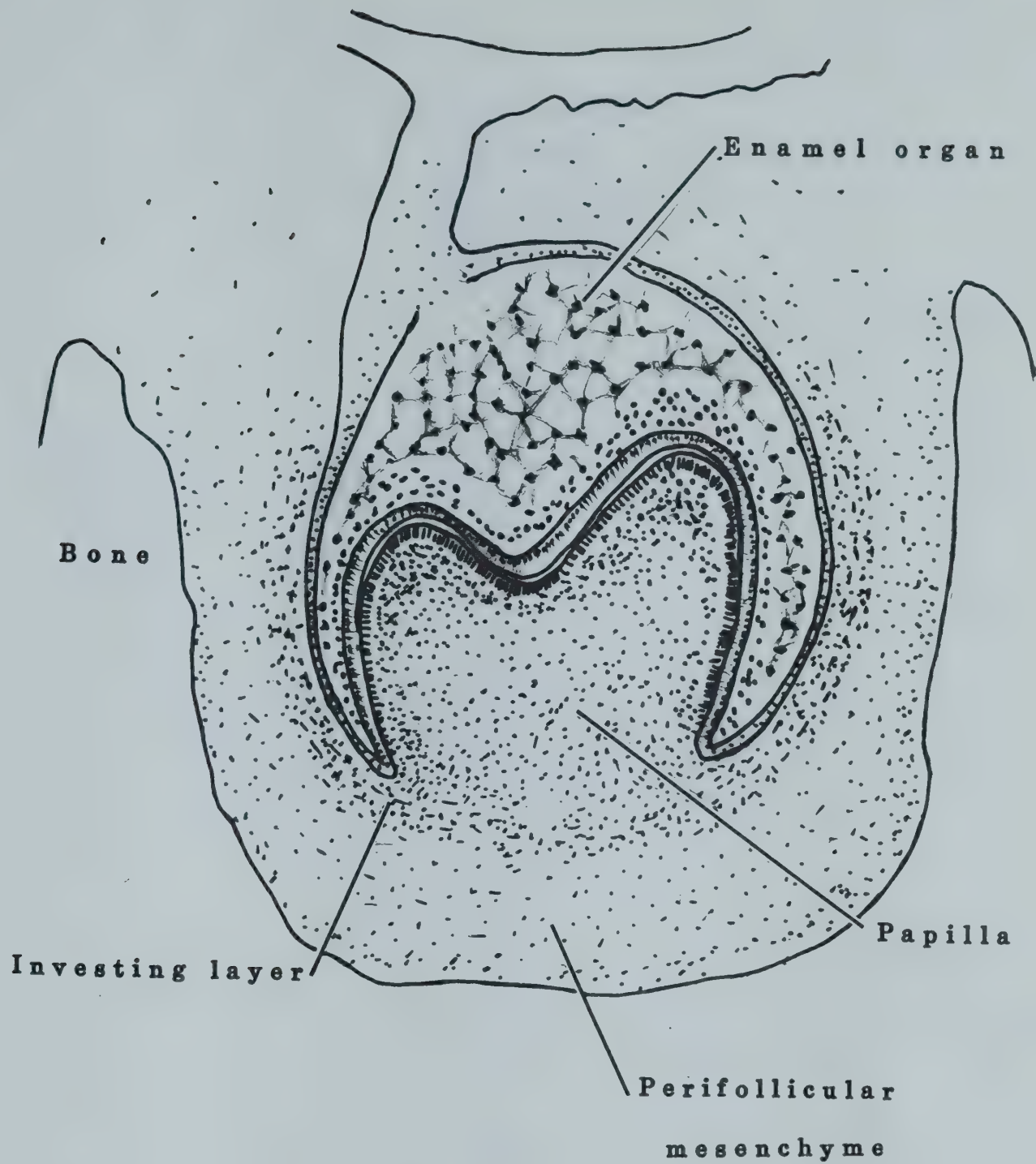


Fig. 11. A developing tooth germ and surrounding follicle.

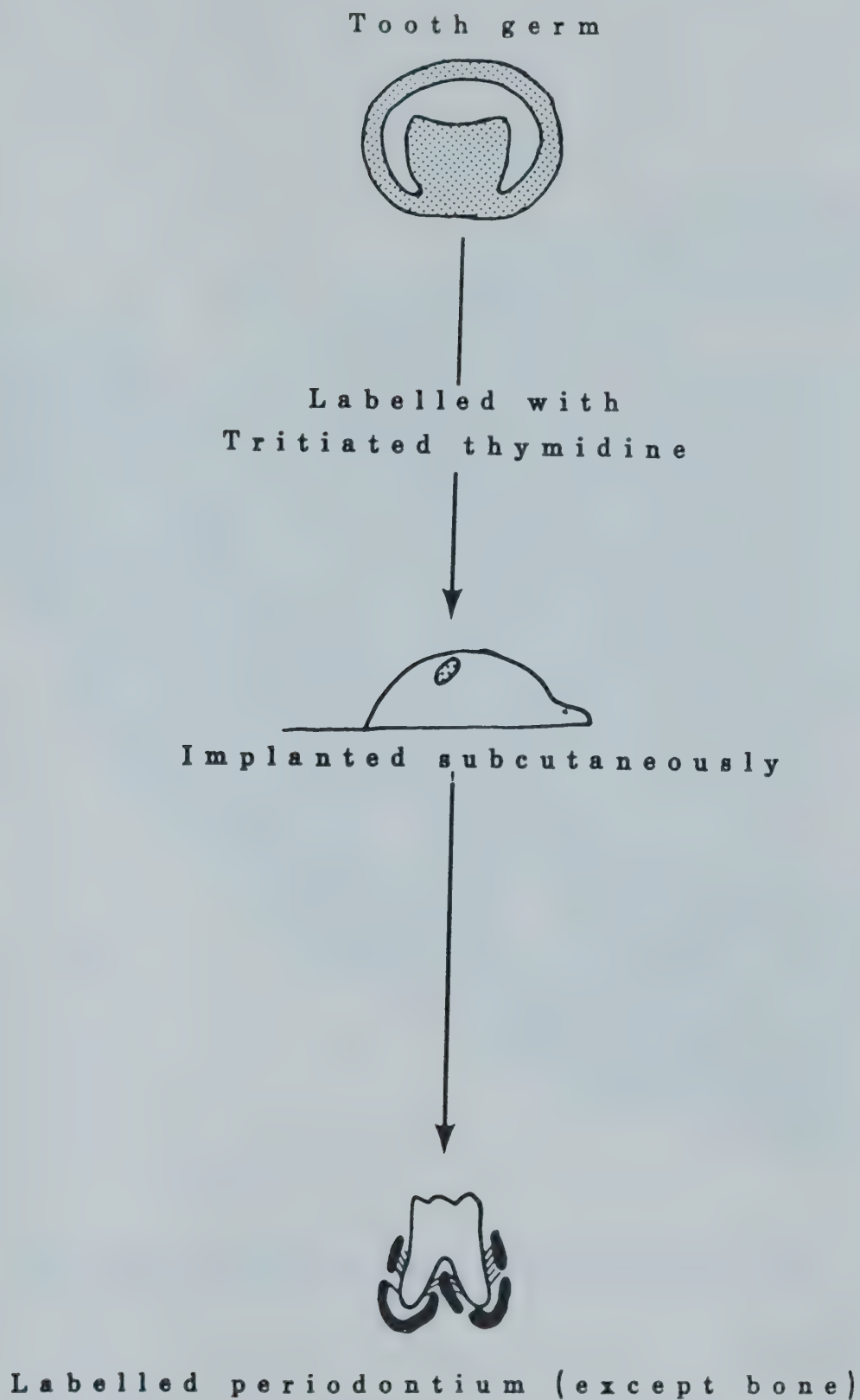


Fig. 12. The transplantation study of Ten Cate et al (1971).

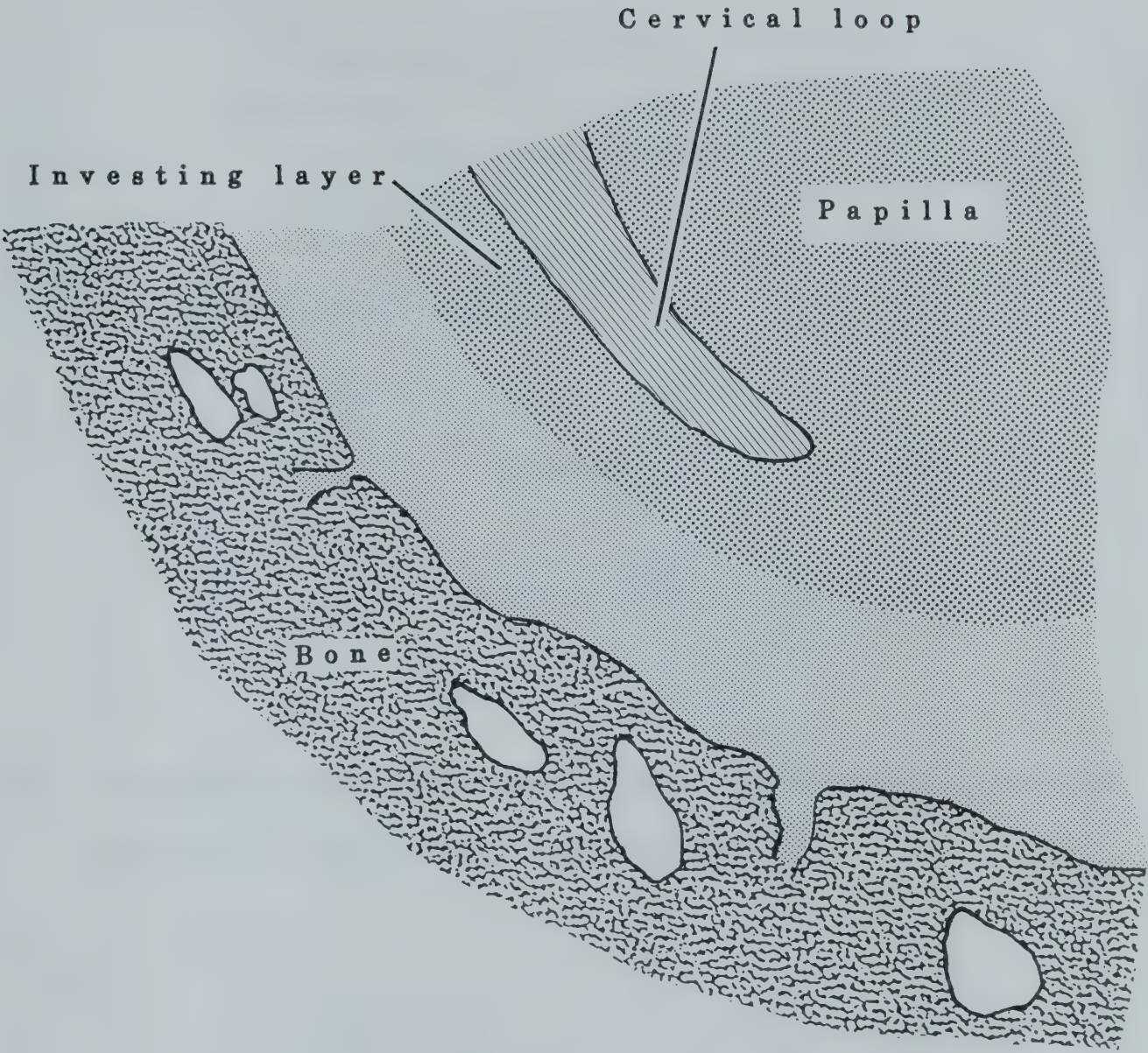


Fig. 13. Diagram showing the continuity between the dental papilla and follicle.

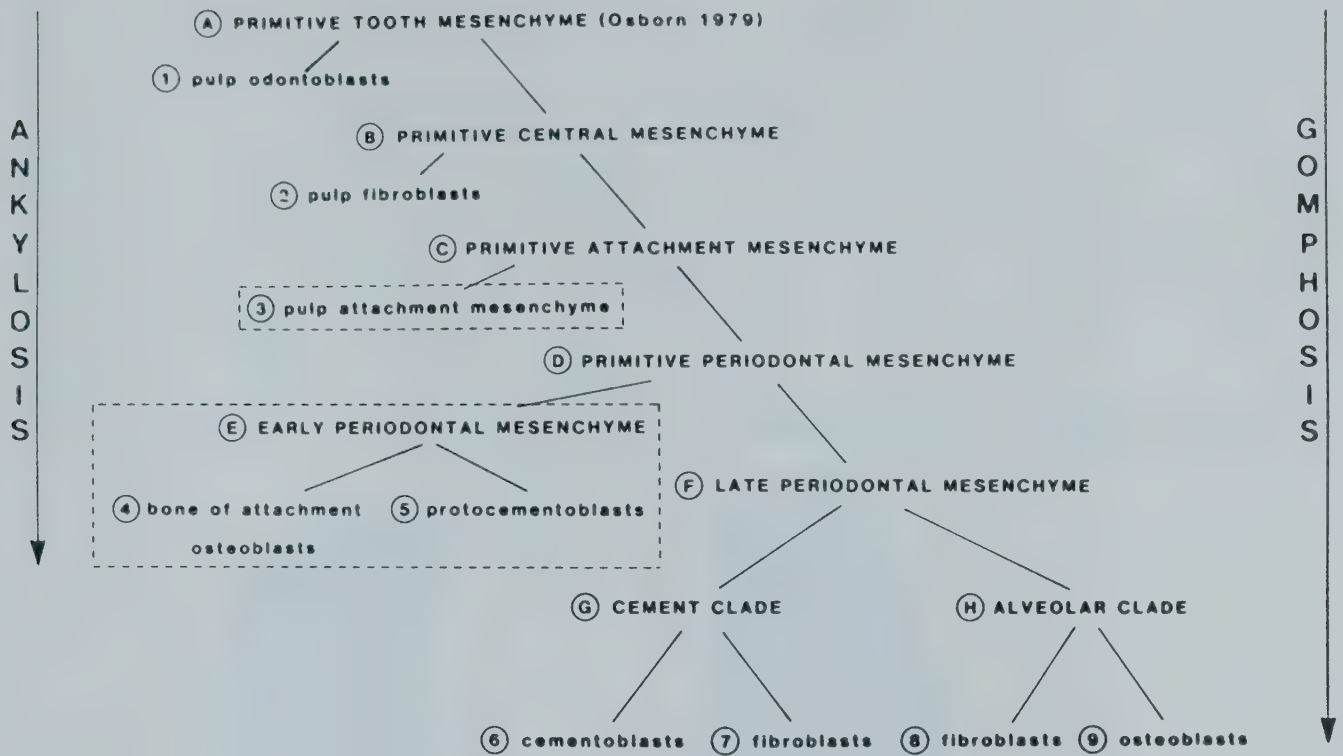


Fig. 14. Cladogram (from Osborn, 1984) showing hypothetical cell lineages which may produce an ankylosis or a gomphosis form of tooth attachment.

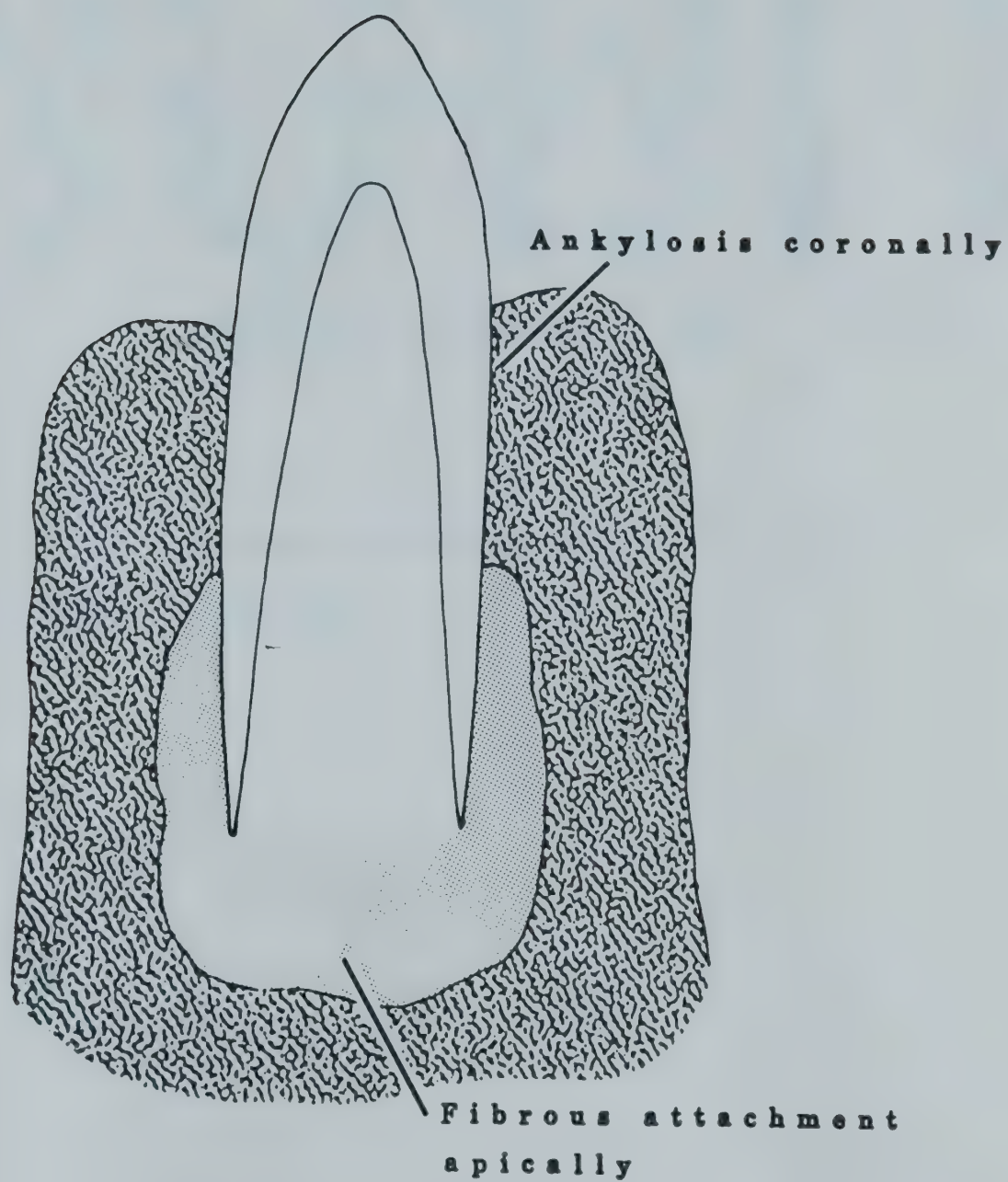


Fig. 15. The tooth attachment seen in Thrinaxodon.

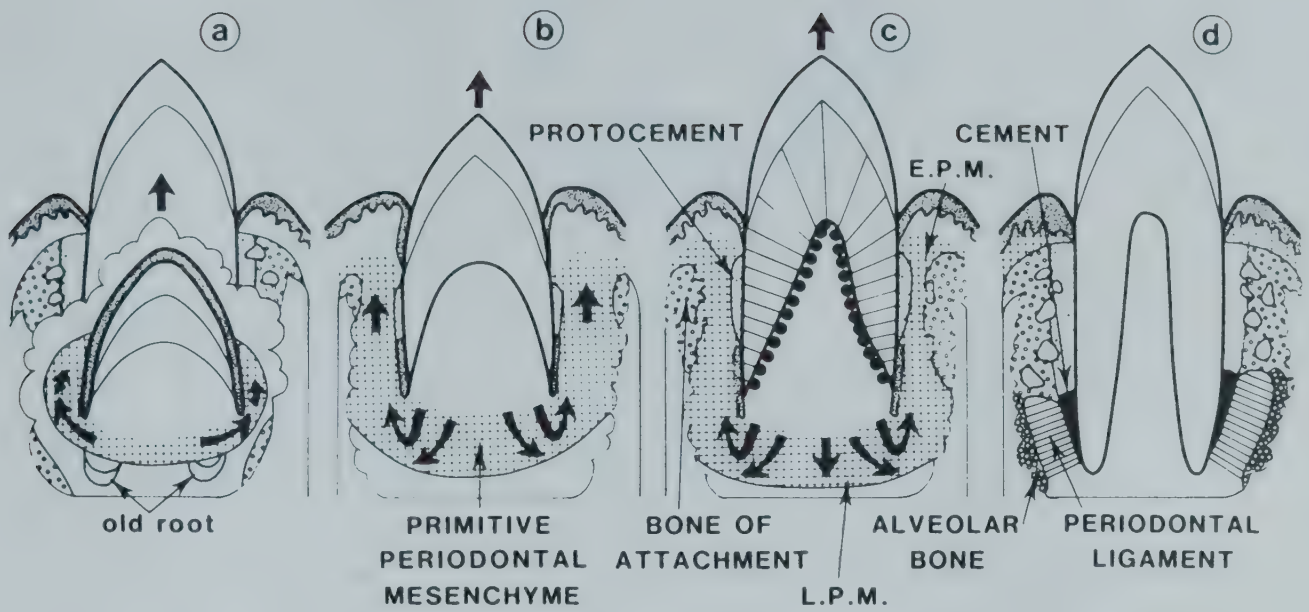
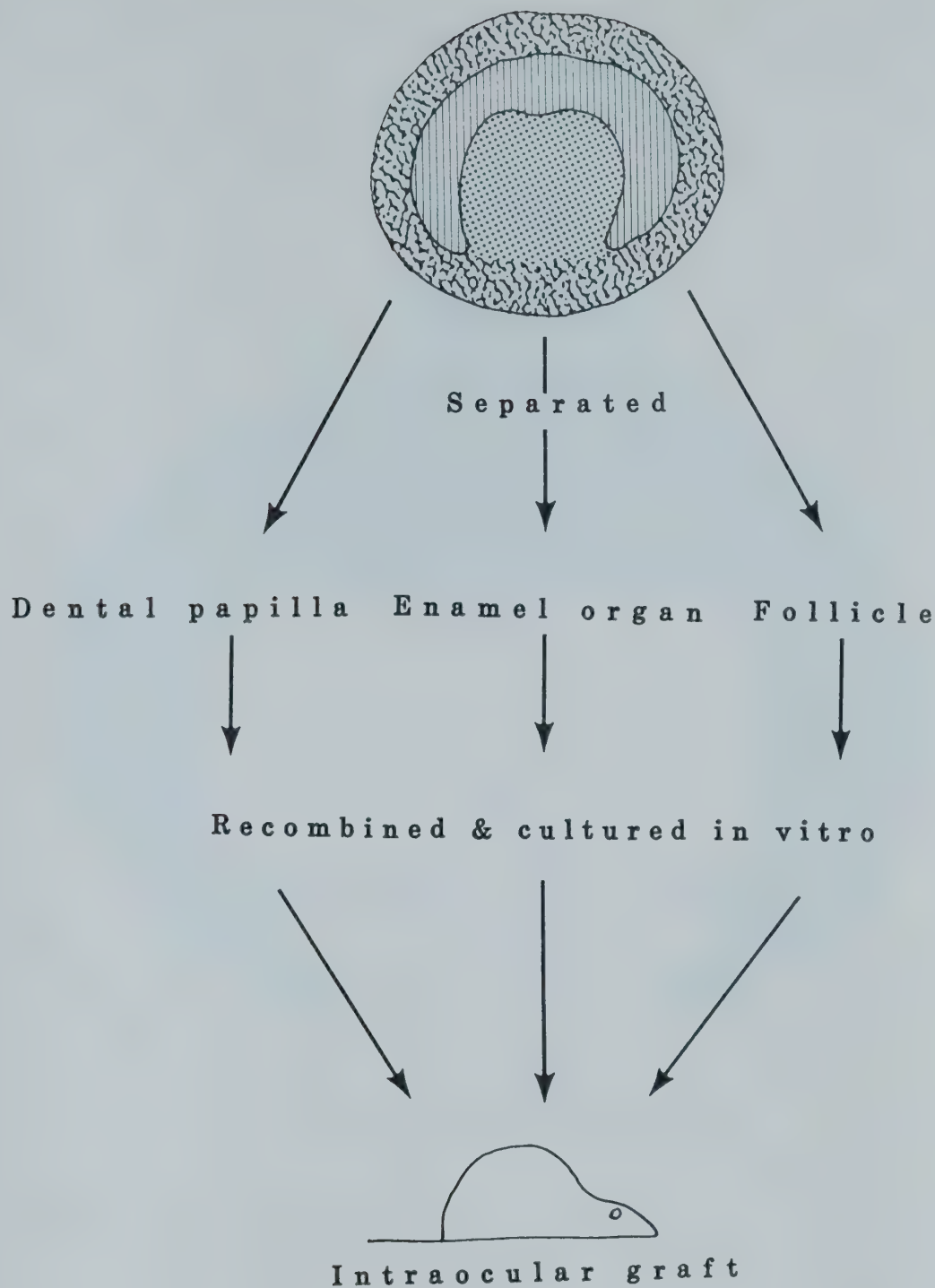


Fig. 16. Osborn's theory of migration of clades in *Thrinaxodon*, producing an ankylosis coronally and a gomphosis apically.

Fig. 17. Yoshikawa and Kollar's study (1981) in which components of tooth germs are recombined and cultured.



RECOMBINATIONS	Tissue age	
	Young	Old
Enamel organ ,Papilla & Follicle	 70 %	 70 %
Enamel organ & Papilla	 66 %	 66 %
Enamel organ & Follicle	 85 %	 15 %

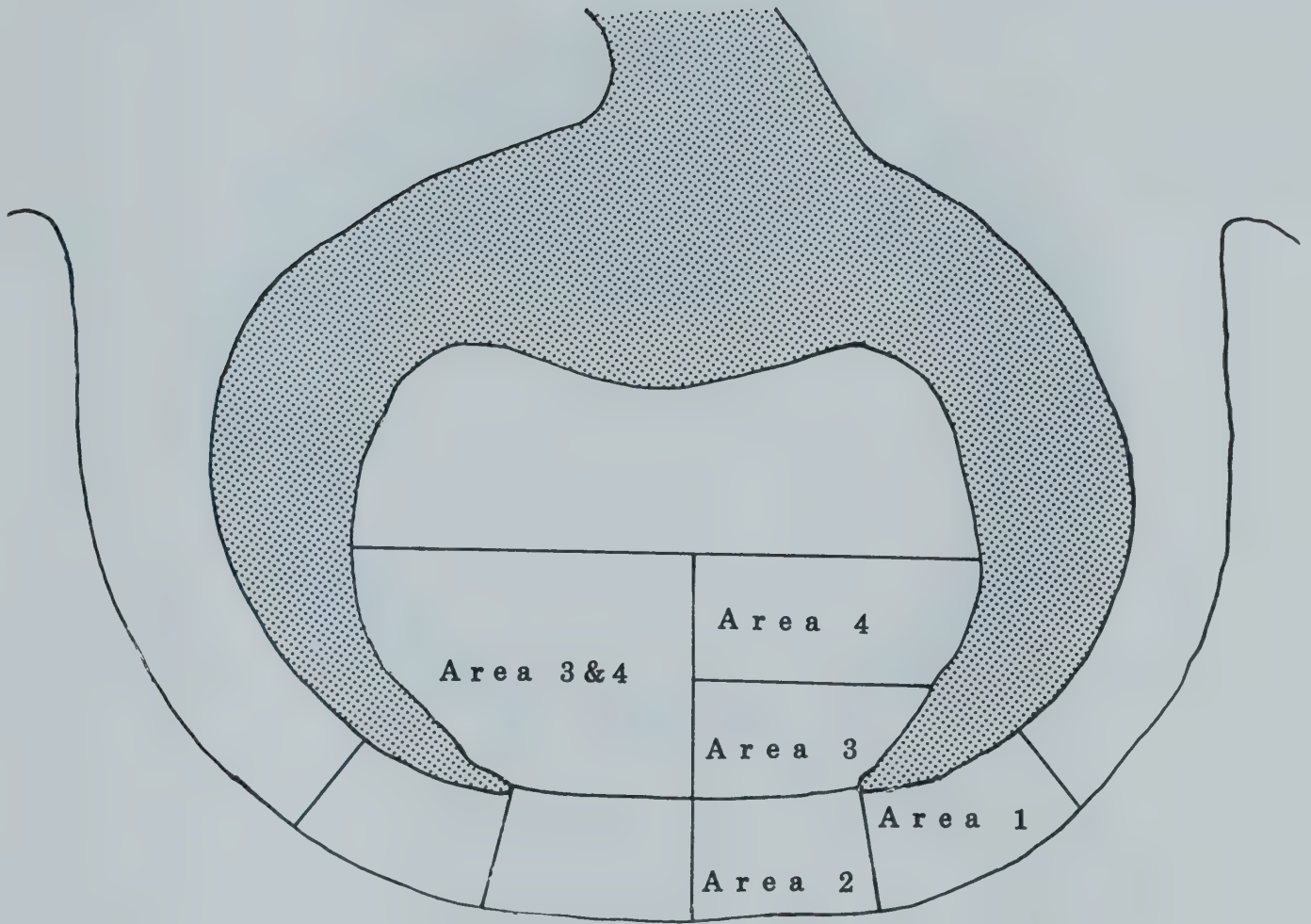


Fig. 18. Diagram of areas 1, 2, 3 and 4 in the tooth germ and dental follicle.

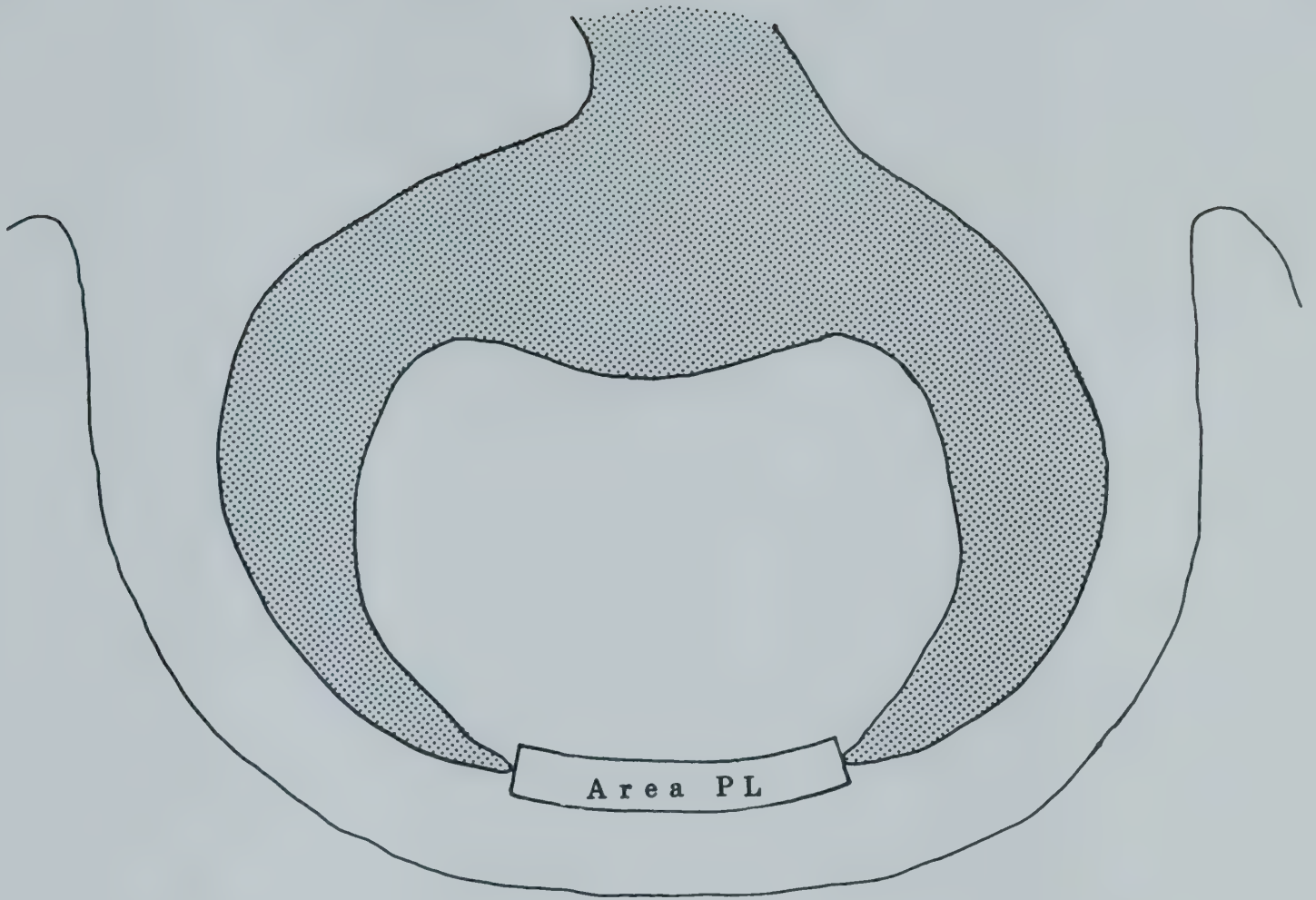
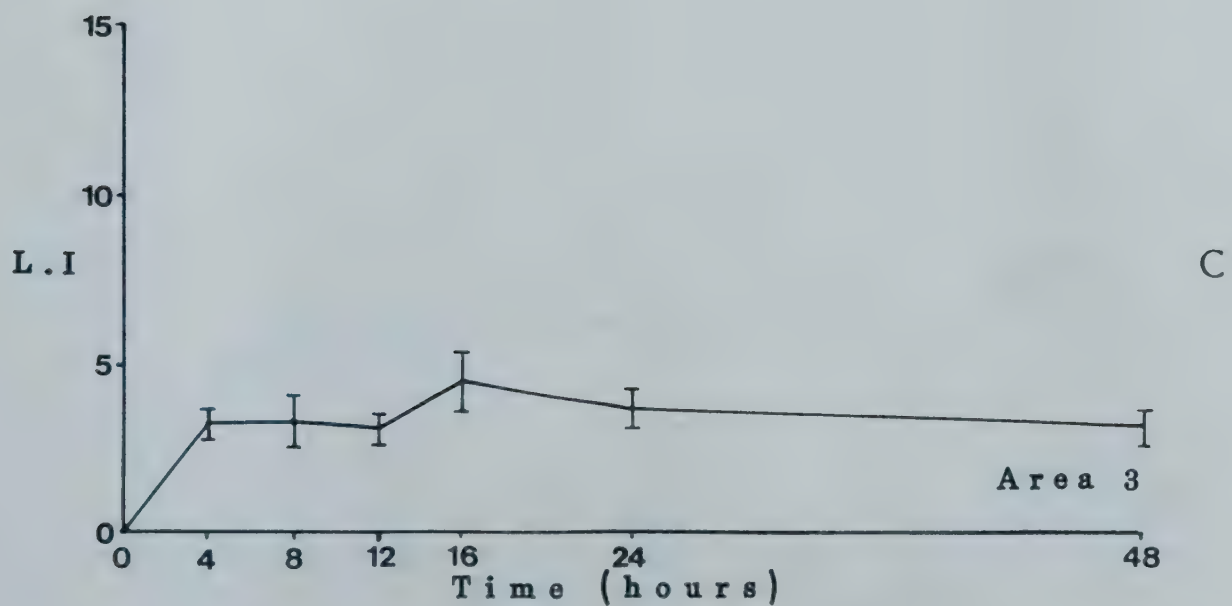
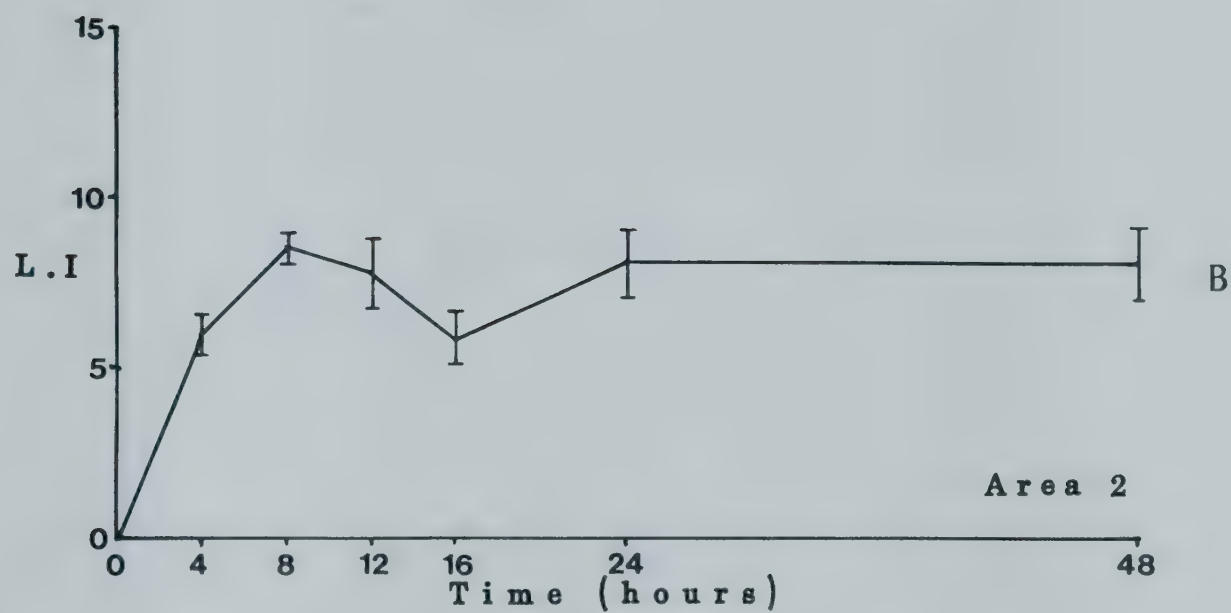
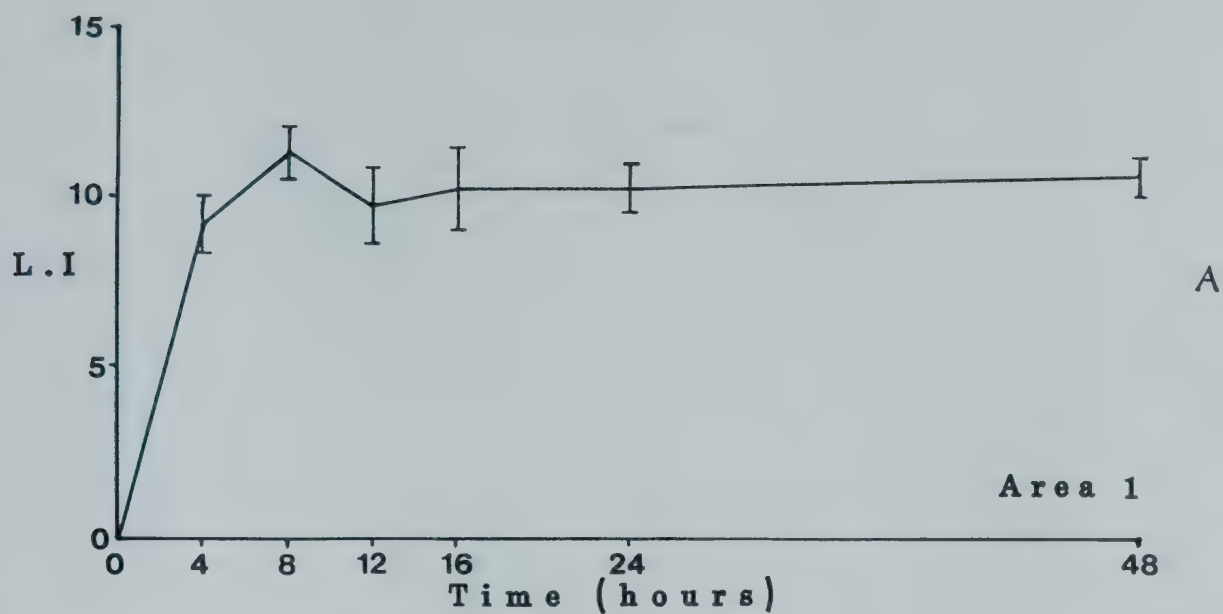


Fig. 19. Diagram of area PL, across the base of the dental papilla.

Fig. 20. Graphs of labelling index against time for 10 day group. A. Area 1; B. Area 2;
C. Area 3; D. Area 4; E. Area 3 & 4; F. Area PL.



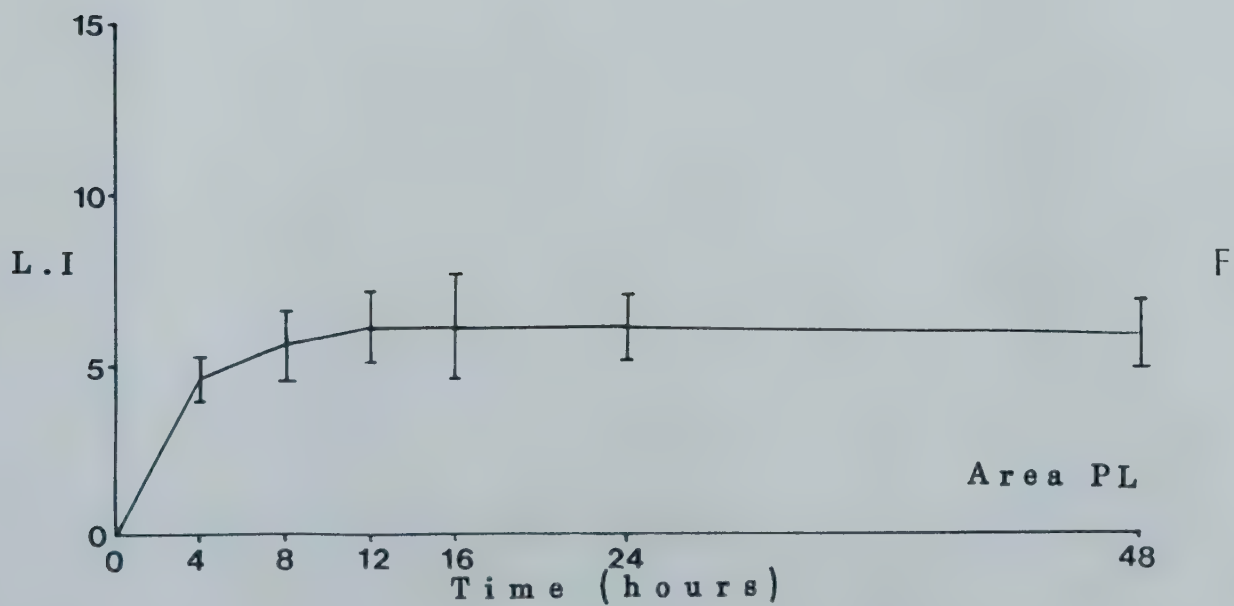
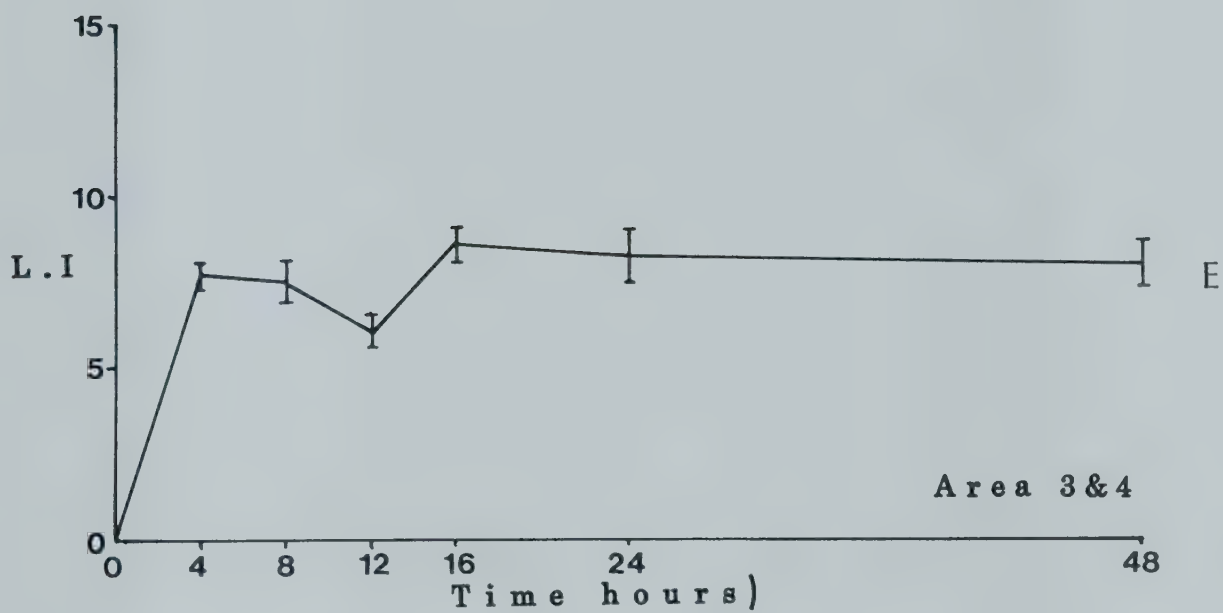
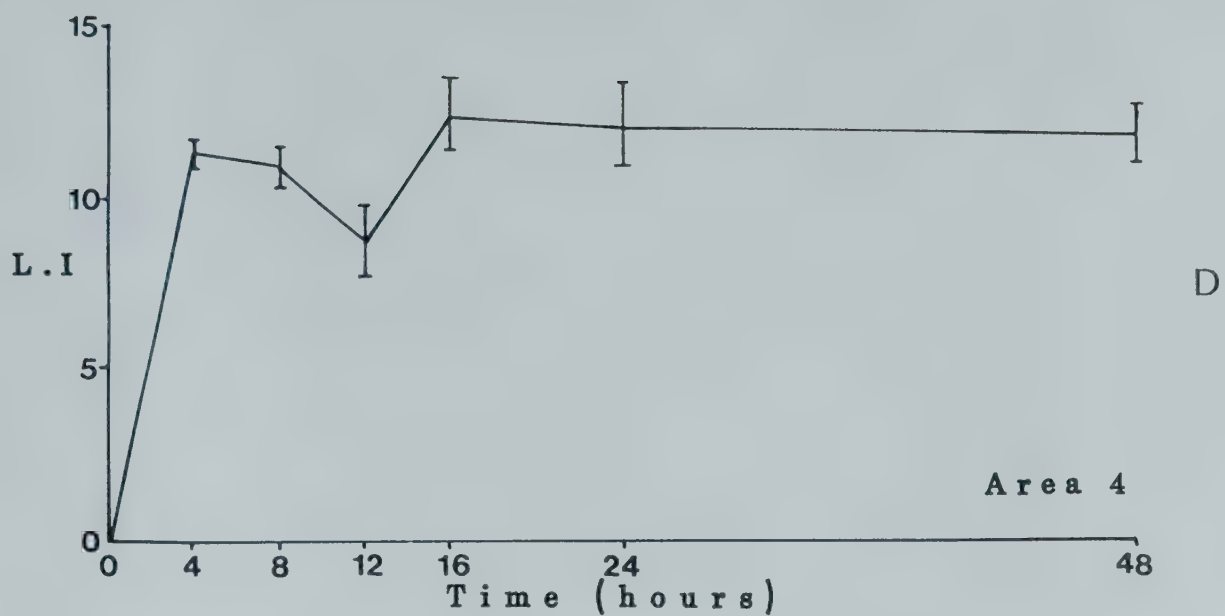
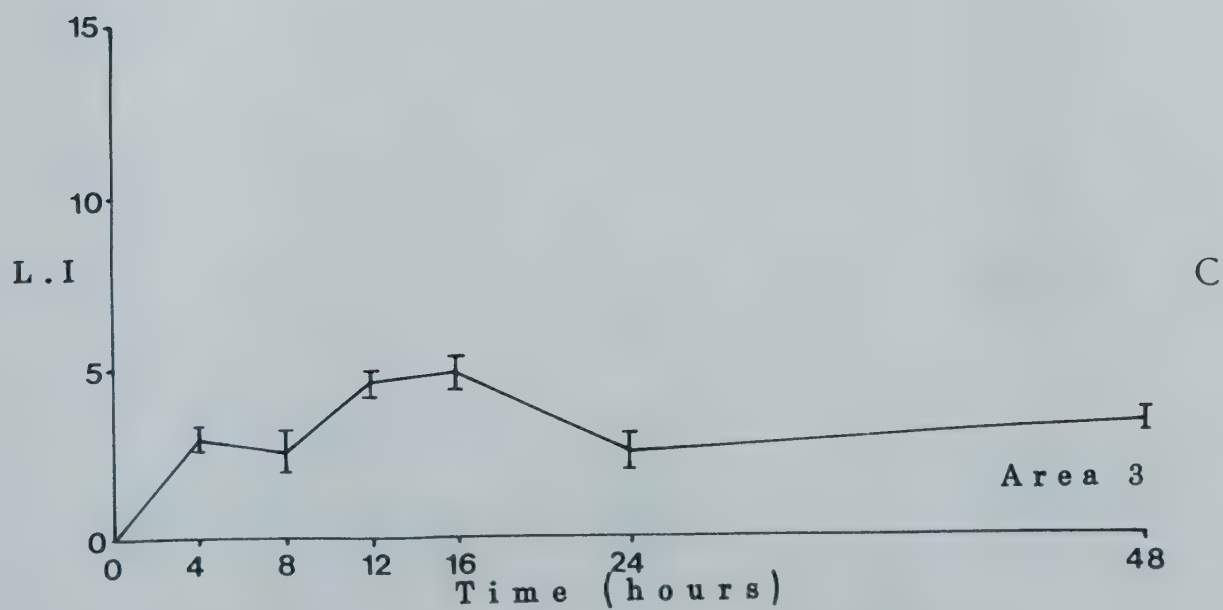
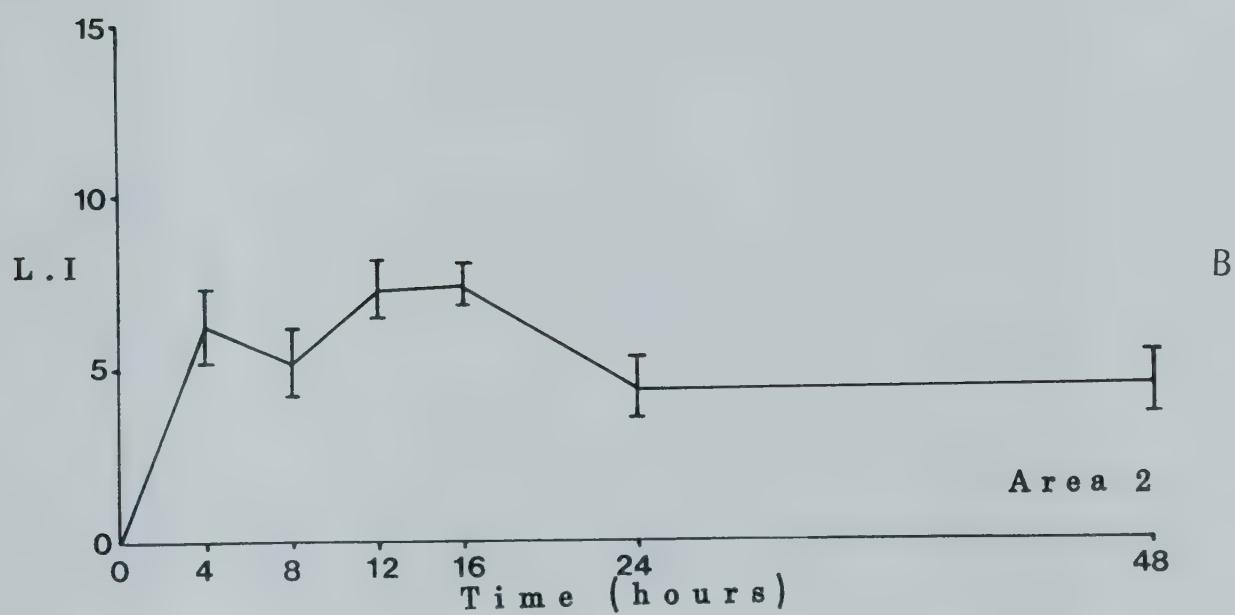
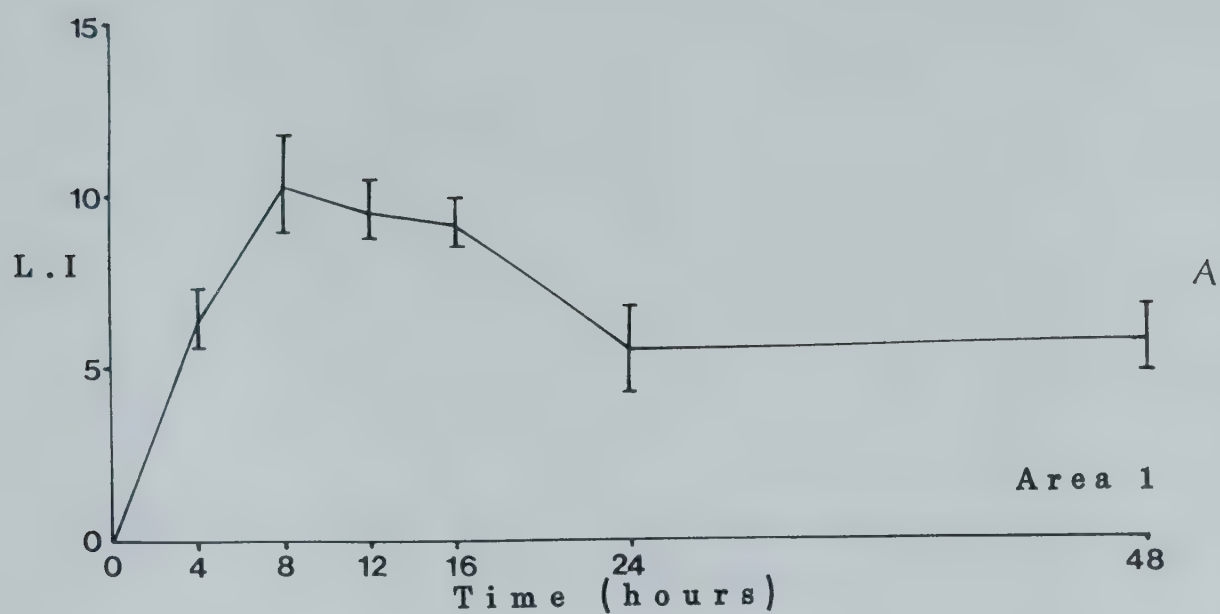


Fig. 21. Graphs of labelling index against time for 13 day group. A. Area 1; B. Area 2;
C. Area 3; D. Area 4; E. Area 3 & 4; F. Area PL.



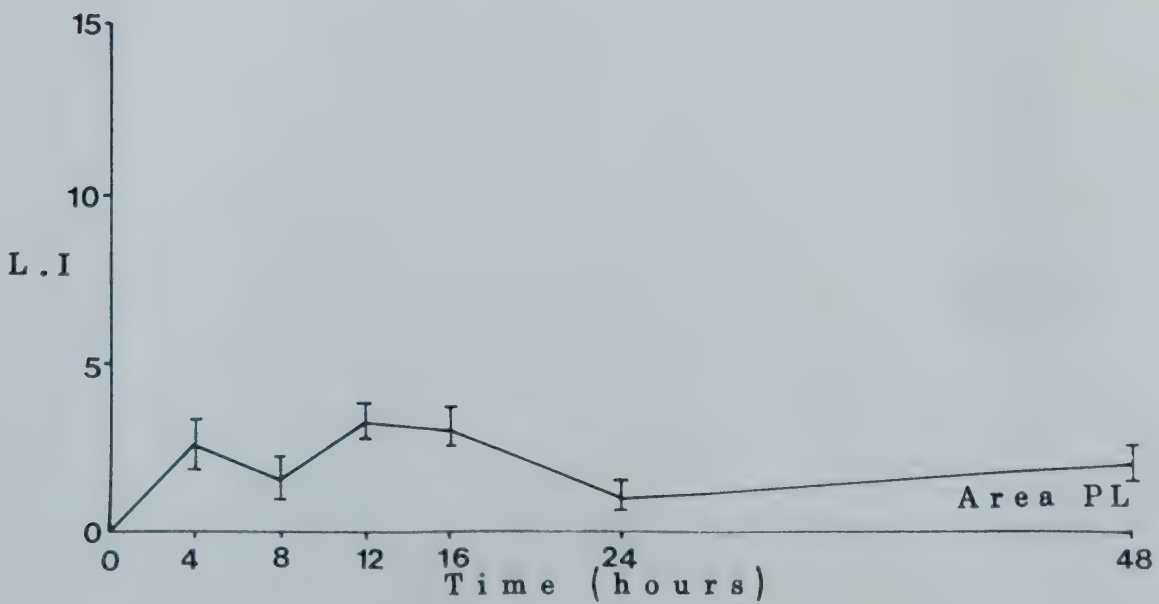
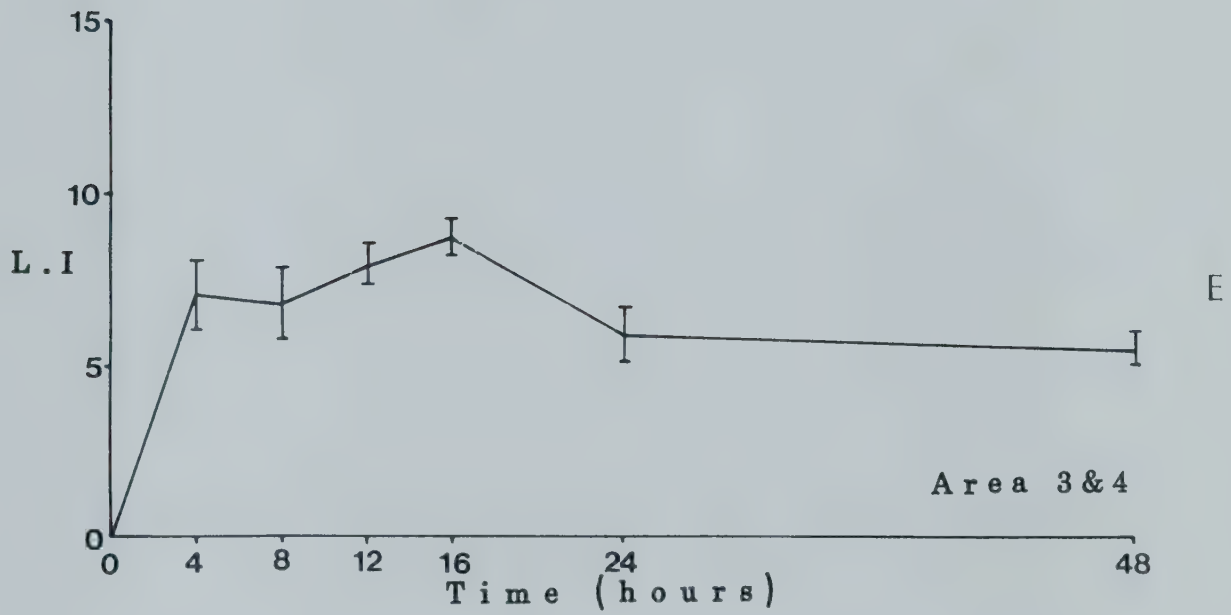
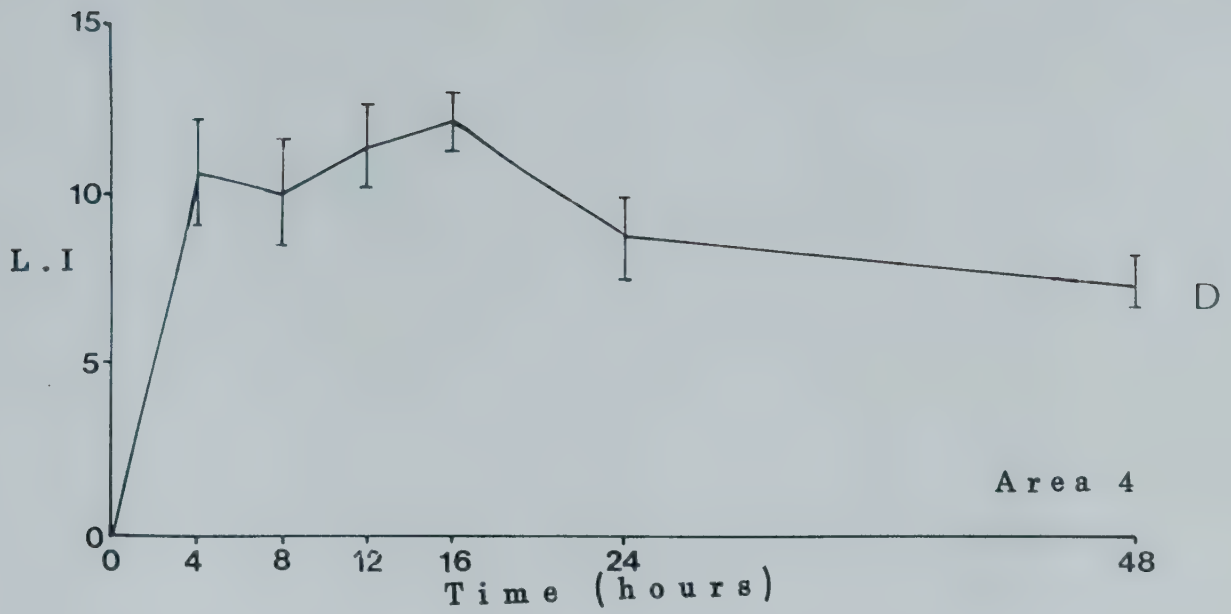
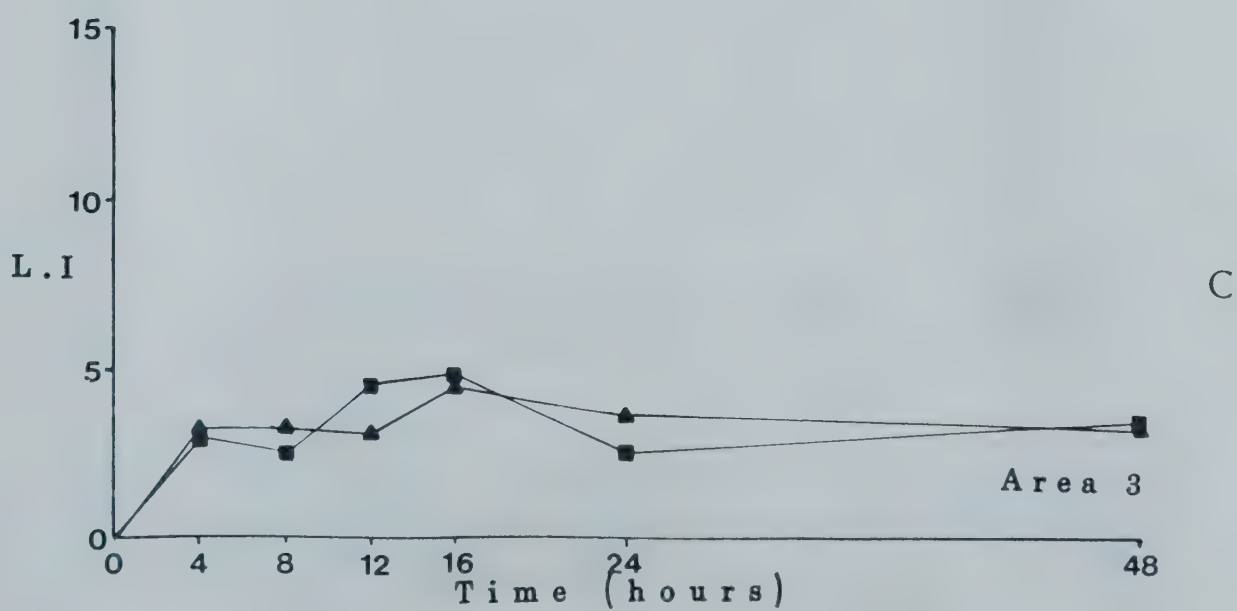
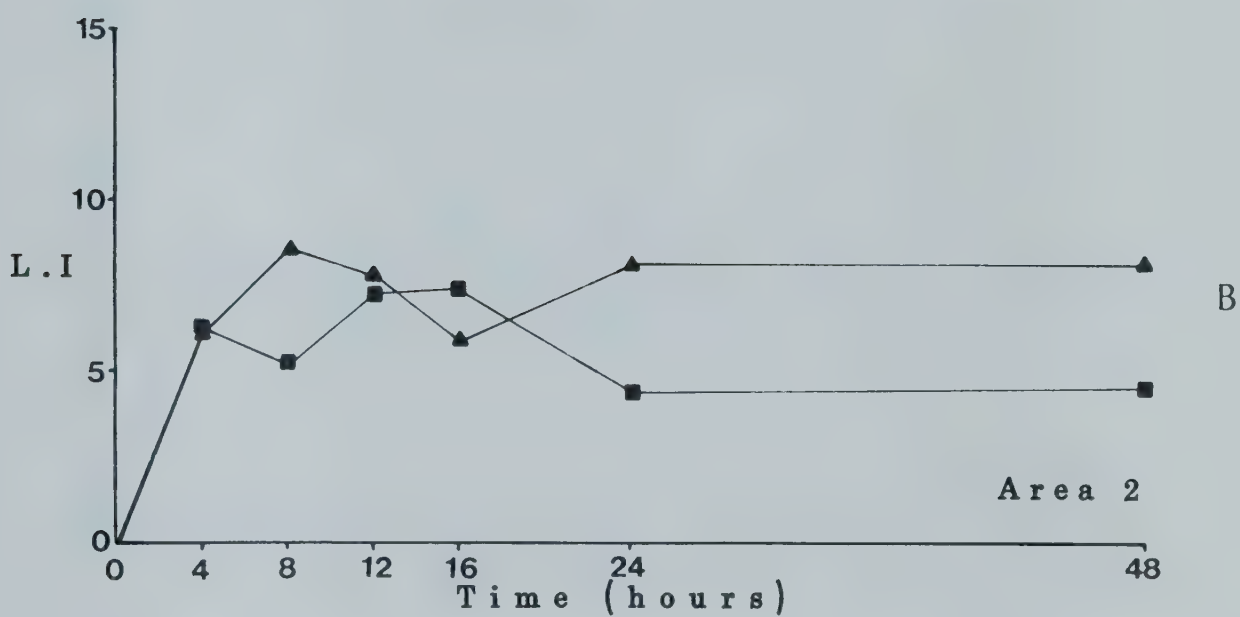
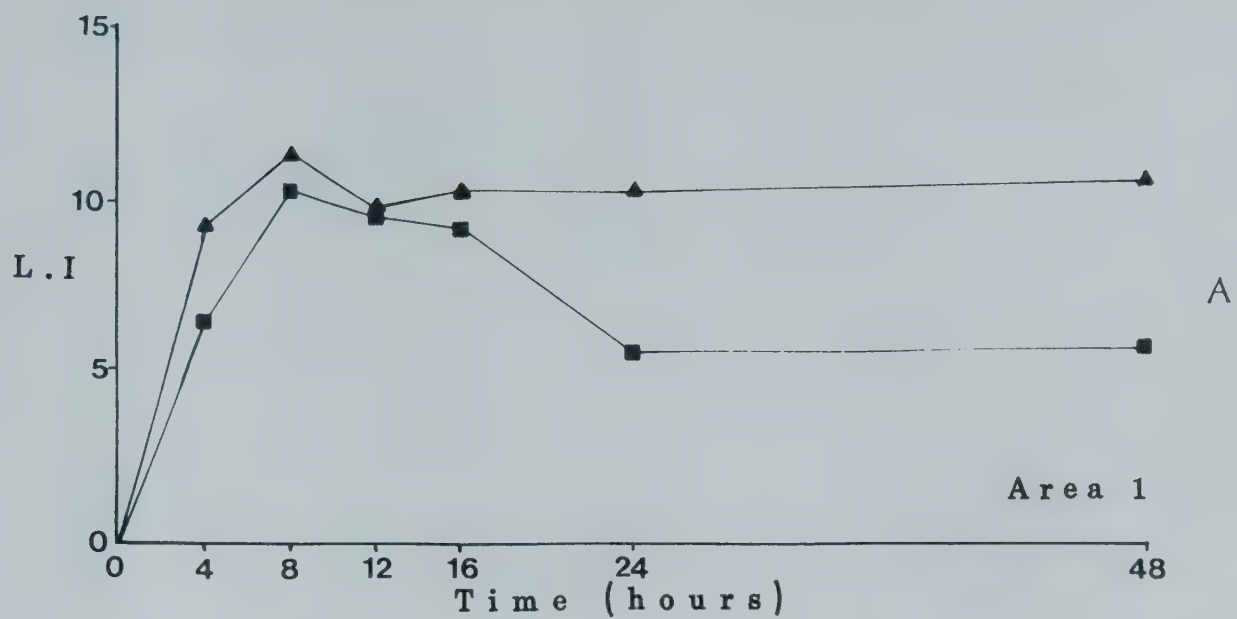
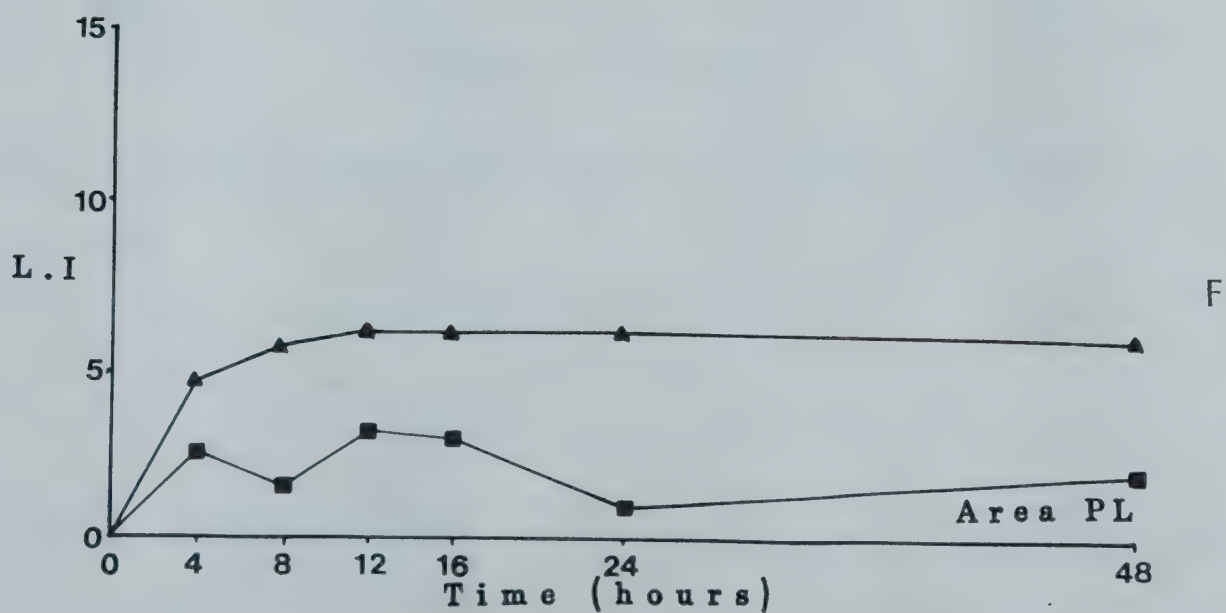
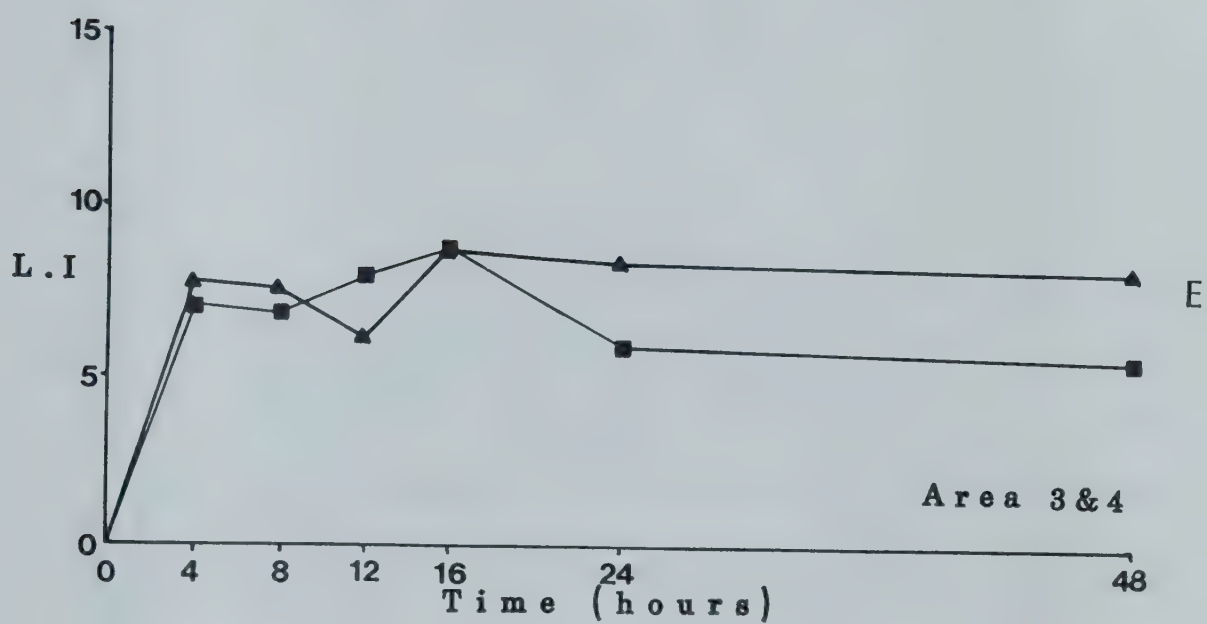
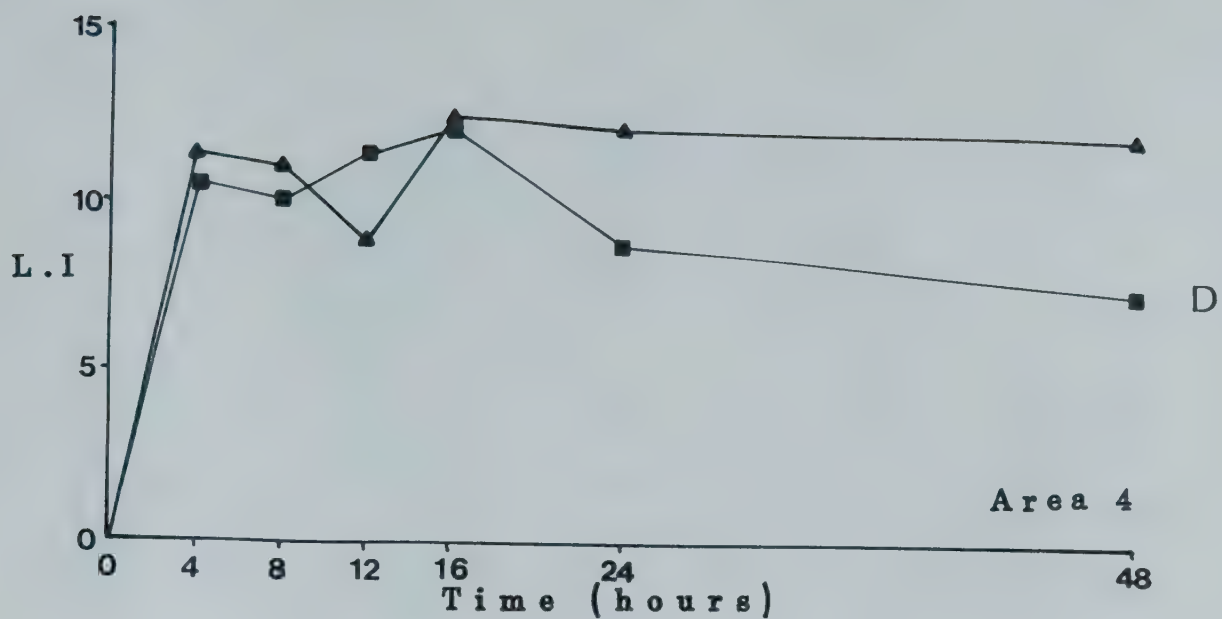


Fig. 22. Comparison between areas of graphs of 10 day and 13 day mice. A. Area 1; B. Area 2;
C. Area 3; D. Area 4; E. Area 3 & 4; F. Area PL.







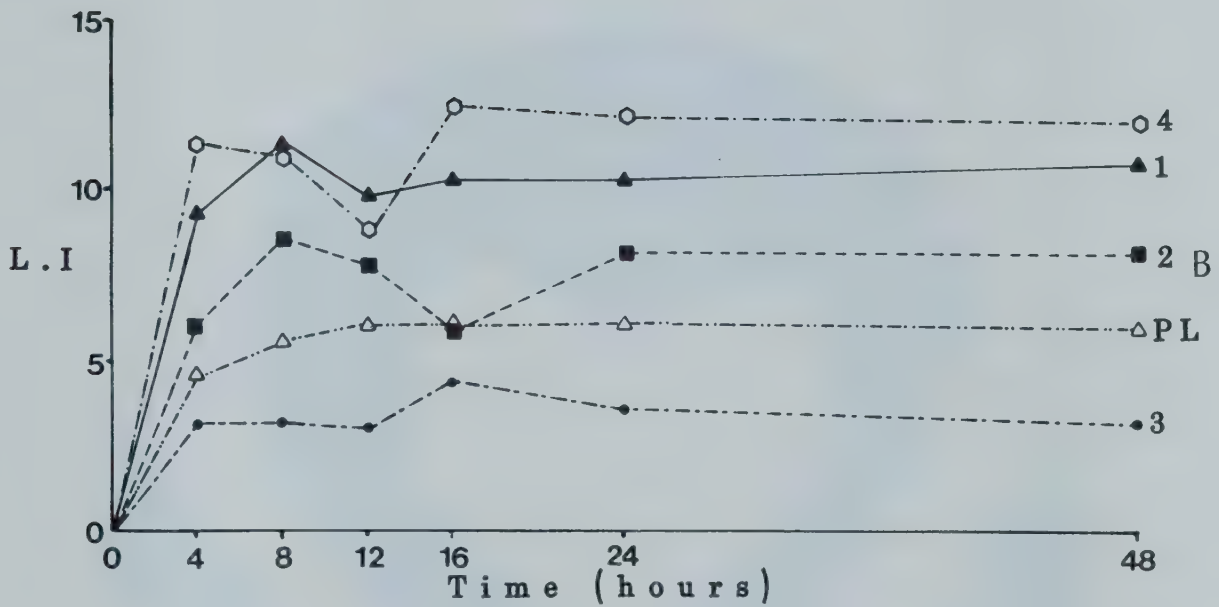
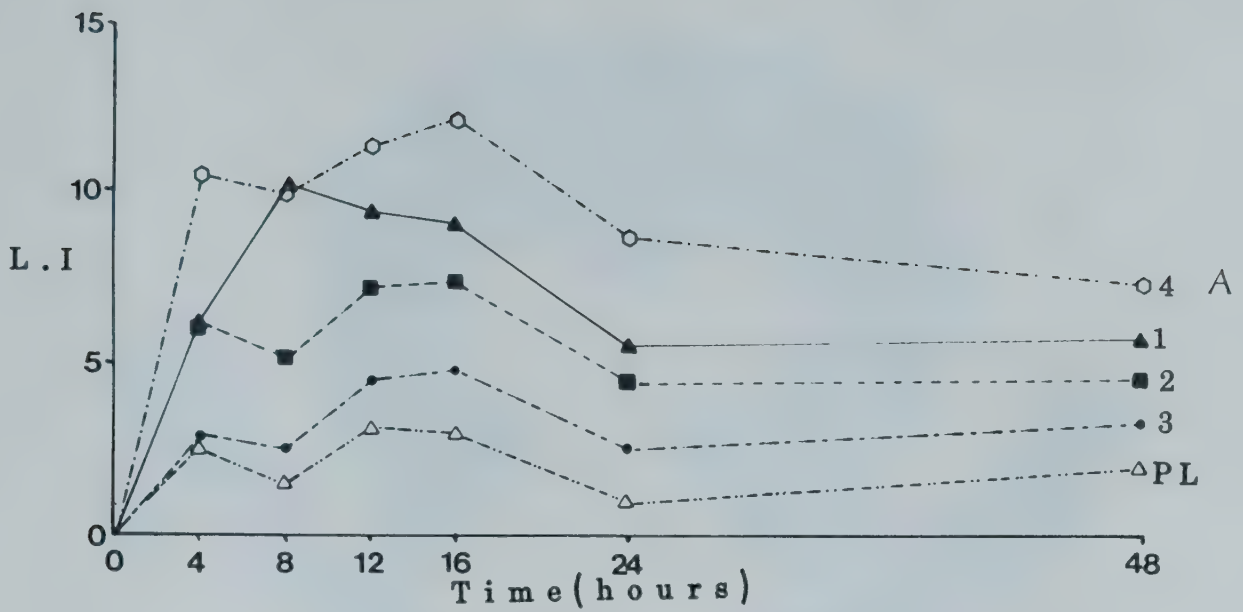


Fig. 23. Comparison of graph shapes of areas 1, 2, 3, 4, and PL in A.13 day mice.B.10 day mice..

- ▲ ——— ▲ Area 1
- - - - ■ Area 2
- - · - · ● Area 3
- - · - · ○ Area 4
- △ - - - △ Area PL

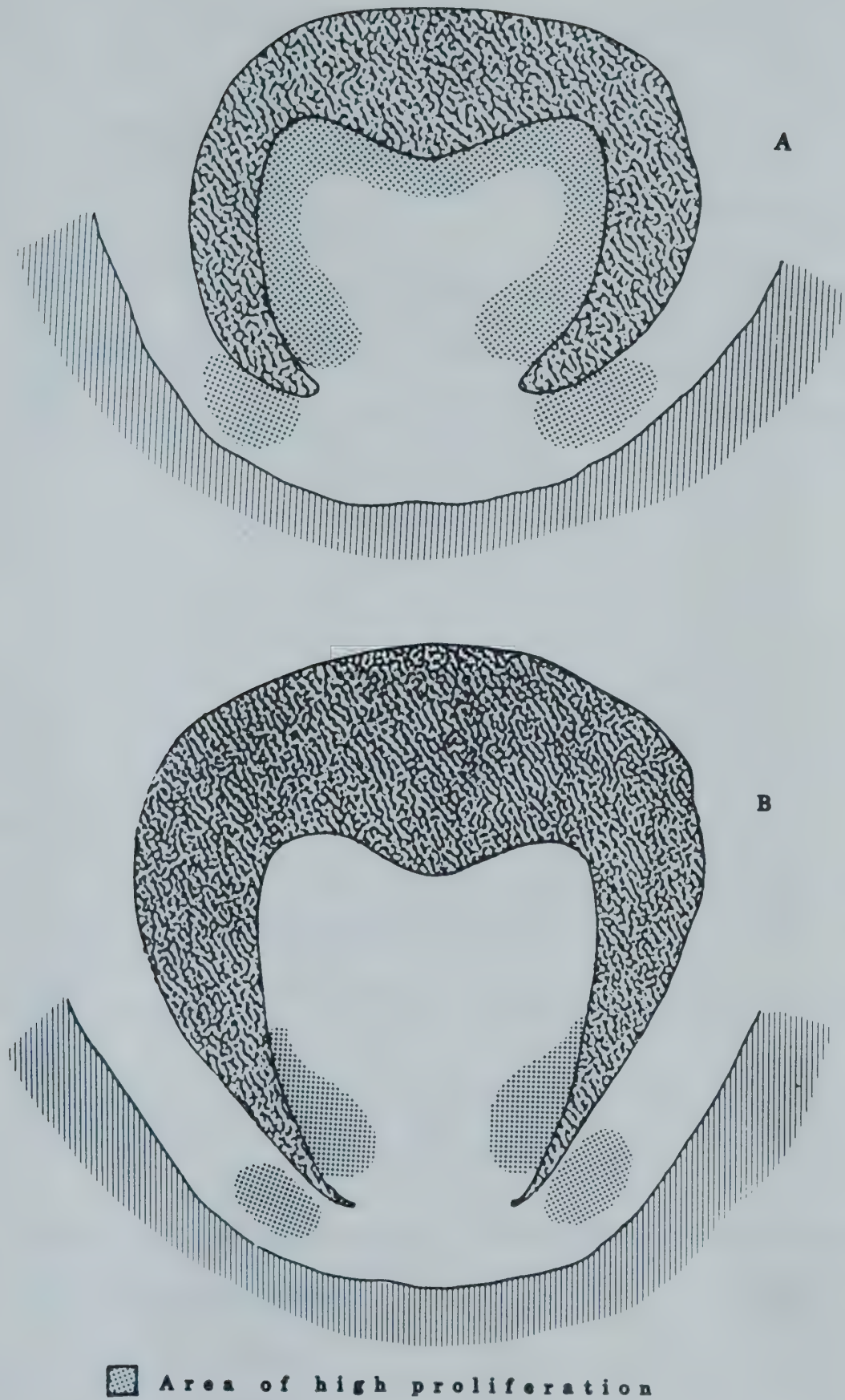


Fig. 24. Sites of cell proliferation seen in A. 10 day tooth germ and follicle. B. 13 day tooth germ and follicle.

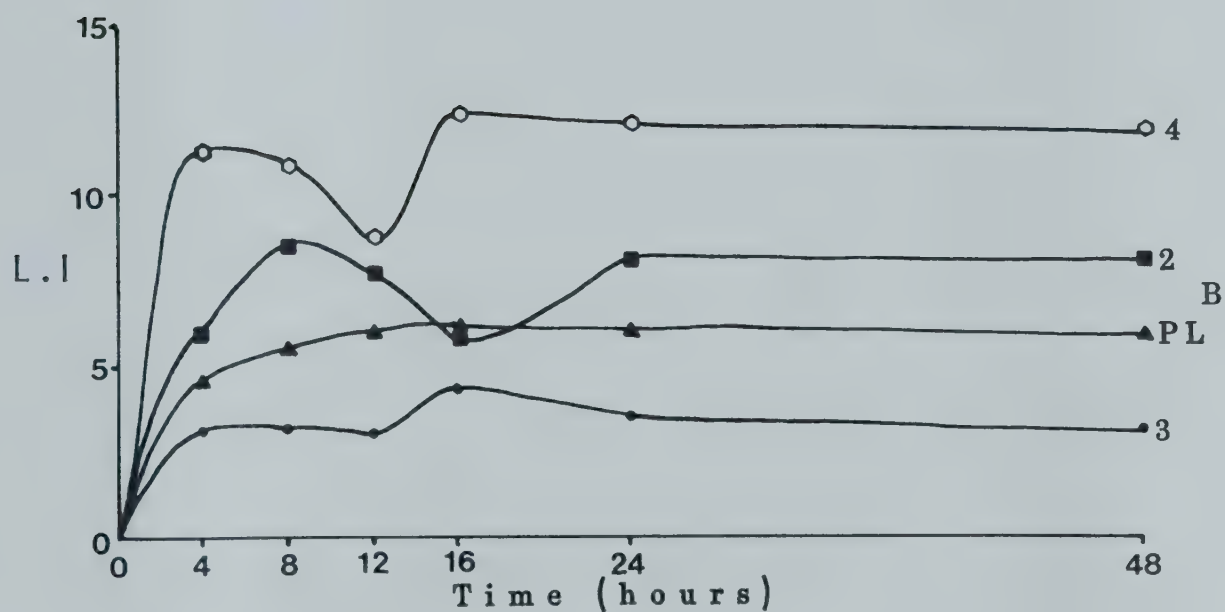
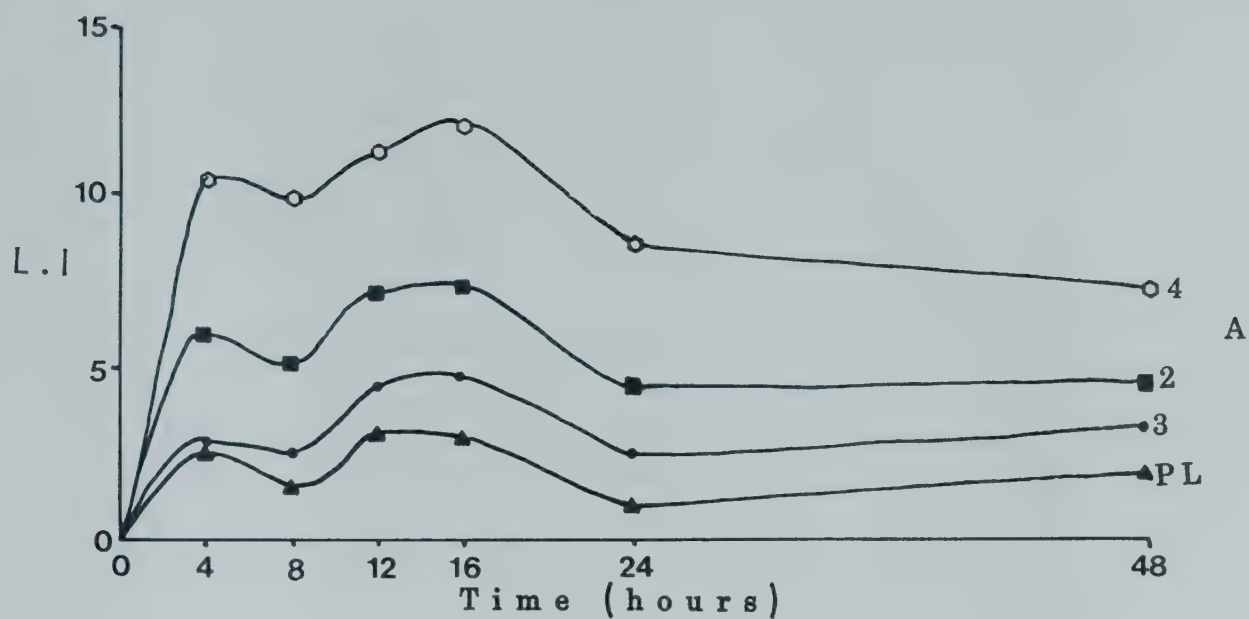


Fig. 25. Comparison of graph shapes of areas 2, 3, 4 and PL in A. 13 day mice; B. 10 day mice.

With curves drawn to fit points.

- — ■ Area 2
- — ● Area 3
- — ○ Area 4
- ▲ — ▲ Area PL

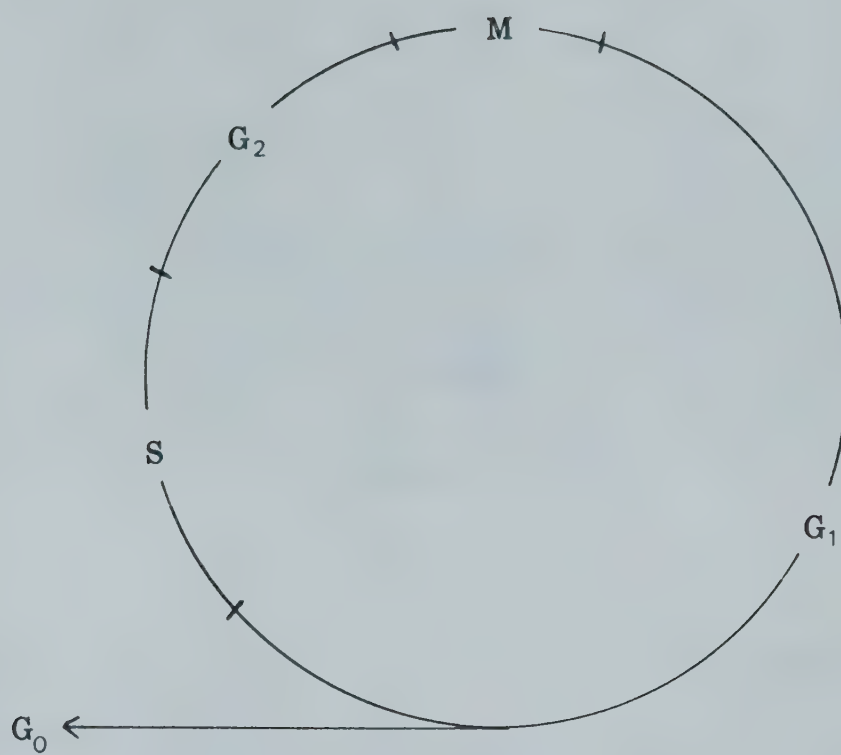


Fig. 26. The mitotic cycle.

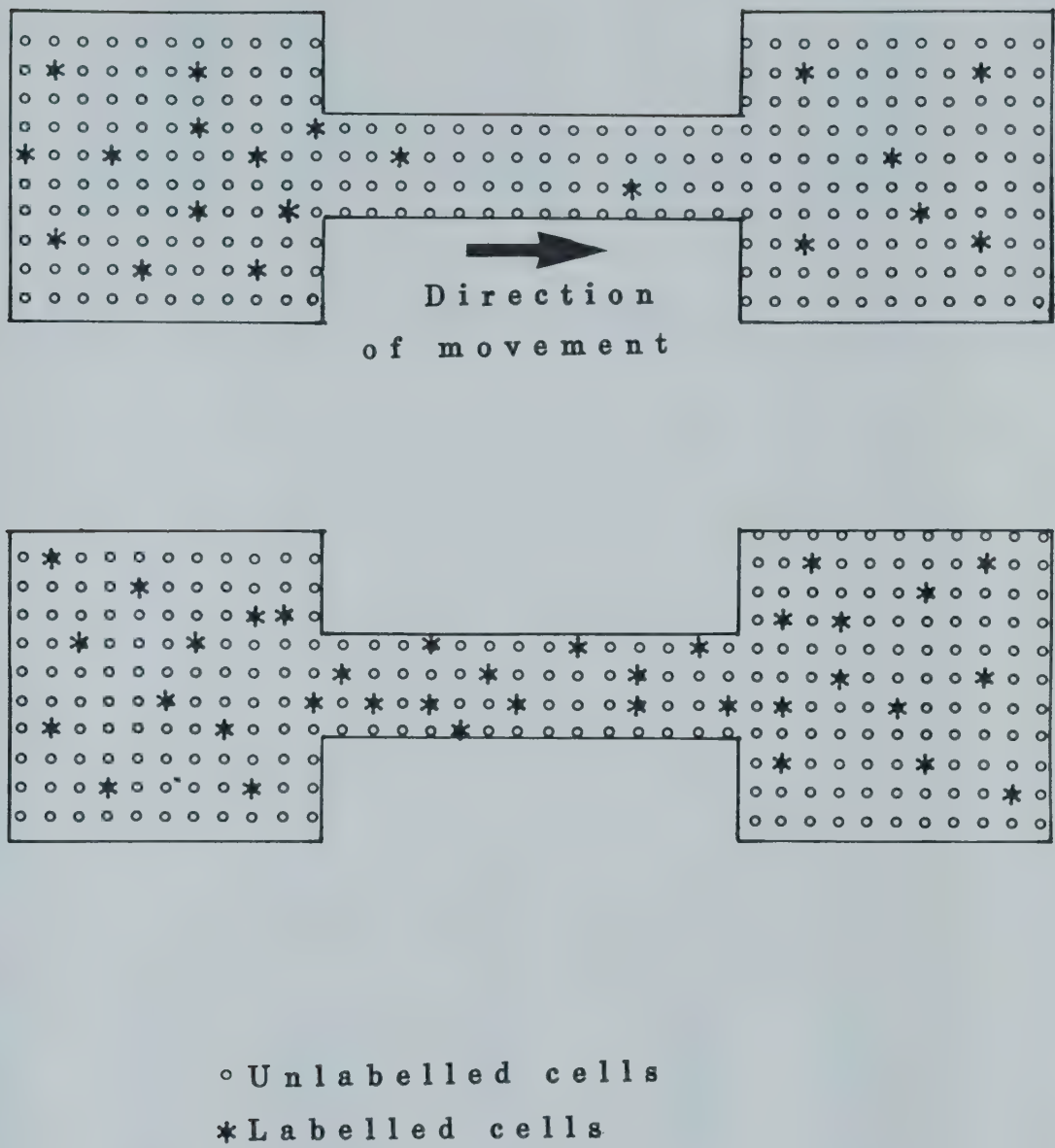


Fig. 27. Diagram showing concept of a 'corridor' between two areas. A. Before steady state.
B. After steady state.

Time Since Injection (hours)	Area 1	Area 2	Area 3	Area 4	Area 3&4	Area PL
	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)
4	9.27 $\pm$ 0.86	6.02 $\pm$ 0.62	3.26 $\pm$ 0.40	11.32 $\pm$ 0.44	7.71 $\pm$ 0.35	4.57 $\pm$ 0.65
8	11.41 $\pm$ 0.75	8.54 $\pm$ 0.44	3.25 $\pm$ 0.76	10.94 $\pm$ 0.57	7.50 $\pm$ 0.61	5.54 $\pm$ 1.04
12	9.79 $\pm$ 1.13	7.79 $\pm$ 0.97	3.05 $\pm$ 0.46	8.76 $\pm$ 1.10	6.06 $\pm$ 0.48	6.09 $\pm$ 1.05
16	10.27 $\pm$ 1.21	5.93 $\pm$ 0.82	4.48 $\pm$ 0.83	12.43 $\pm$ 1.05	8.60 $\pm$ 0.46	6.08 $\pm$ 1.57
24	10.28 $\pm$ 0.66	8.12 $\pm$ 0.93	3.68 $\pm$ 0.54	12.13 $\pm$ 1.20	8.23 $\pm$ 0.78	6.14 $\pm$ 0.97
48	10.69 $\pm$ 0.54	8.09 $\pm$ 1.00	3.11 $\pm$ 0.52	11.89 $\pm$ 0.82	8.01 $\pm$ 0.66	5.92 $\pm$ 1.00

TABLE 1. Results of 10 day old mice, mean L.I. ($\pm$ Standard error) at different times for each area.

t Test Between L.I. at times (hours)	Area 1	Area 2	Area 3	Area 4	Area 3&4	Area PL
	p	p	p	p	p	p
4—8	0.11	0.01*	0.99	0.61	0.75	0.42
8—12	0.25	0.50	0.83	0.10	0.09	0.71
12—16	0.78	0.20	0.13	0.04*	0.004**	0.99
16—24	0.99	0.15	0.41	0.87	0.75	0.98
24—48	0.64	0.98	0.46	0.87	0.83	0.88
4—12	—	—	—	—	0.012*	—
8—16	—	0.01*	—	—	—	—

TABLE 2. Comparison of L.I. between times for 10 day mice using Student's t test.

- * $p < 0.05$
 ** $p < 0.01$
 *** $p < 0.001$

Time Since Injection (hours)	Area 1	Area 2	Area 3	Area 4	Area 3&4	Area PL
	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)	Mean L.I. ($\pm$ S.E.)
4	6.42 $\pm$ 0.89	6.28 $\pm$ 1.07	2.95 $\pm$ 0.34	10.62 $\pm$ 1.56	7.09 $\pm$ 0.99	2.64 $\pm$ 0.77
8	10.31 $\pm$ 1.42	5.21 $\pm$ 1.02	2.60 $\pm$ 0.59	10.08 $\pm$ 1.56	6.80 $\pm$ 1.03	1.58 $\pm$ 0.67
12	9.61 $\pm$ 0.86	7.34 $\pm$ 0.85	4.57 $\pm$ 0.40	11.44 $\pm$ 1.2	7.97 $\pm$ 0.59	3.31 $\pm$ 0.54
16	9.21 $\pm$ 0.70	7.45 $\pm$ 0.64	4.85 $\pm$ 0.47	12.17 $\pm$ 0.79	8.77 $\pm$ 0.53	3.19 $\pm$ 0.58
24	5.51 $\pm$ 1.20	4.44 $\pm$ 0.91	2.61 $\pm$ 0.55	8.71 $\pm$ 1.22	5.92 $\pm$ 0.80	1.14 $\pm$ 0.43
48	5.76 $\pm$ 0.91	4.54 $\pm$ 0.96	3.46 $\pm$ 0.29	7.44 $\pm$ 0.77	5.55 $\pm$ 0.45	2.10 $\pm$ 0.55

TABLE 3. Results of 13 day old mice, mean L.I. ($\pm$ Standard error) at different times for each area.

t Test Between L.I. at times (hours)	Area 1	Area 2	Area 3	Area 4	Area 3&4	Area PL
	p	p	p	p	p	p
4—8	0.05	0.49	0.64	0.82	0.85	0.32
8—12	0.66	0.13	0.01*	0.49	0.31	0.06
12—16	0.72	0.92	0.66	0.61	0.33	0.88
16—24	0.01*	0.01*	0.008**	0.03*	0.007**	0.02*
24—48	0.87	0.94	0.18	0.38	0.68	0.20
8—16	—	0.07	—	—	0.08	—

TABLE 4. Comparison of L.I. between times for 13 day mice using Student's t test.

- * p<0.05
 ** p<0.01
 *** p<0.001

t Test Between 10 day & 13 day at times (hours)	Area 1	Area 2	Area 3	Area 4	Area 3&4	Area PL
	p	p	p	p	p	p
4	0.07	0.83	0.65	0.57	0.46	0.10
8	0.48	0.009**	0.52	0.59	0.56	0.01*
12	0.89	0.73	0.02*	0.12	0.03*	0.02*
16	0.43	0.18	0.68	0.85	0.84	0.05*
24	0.02*	0.02*	0.19	0.07	0.06	0.0006***
48	0.0004***	0.002**	0.56	0.001**	0.008**	0.004**

TABLE 5. Comparison of 10 day and 13 day results using Student's t test.

- * $p < 0.05$
- ** $p < 0.01$
- *** $p < 0.001$

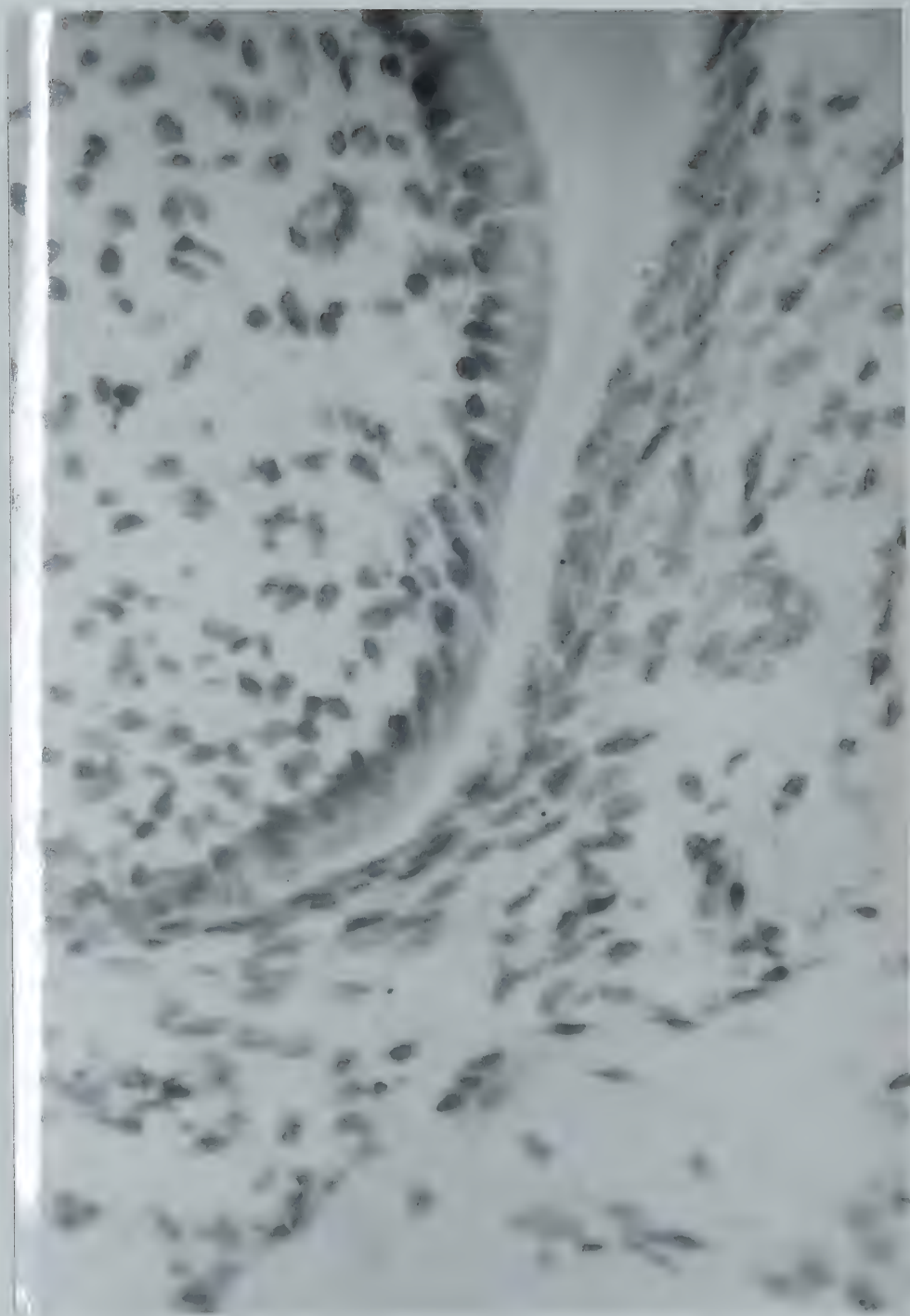


Plate 1 Control - unlabelled mouse, showing background grain count. (x600)

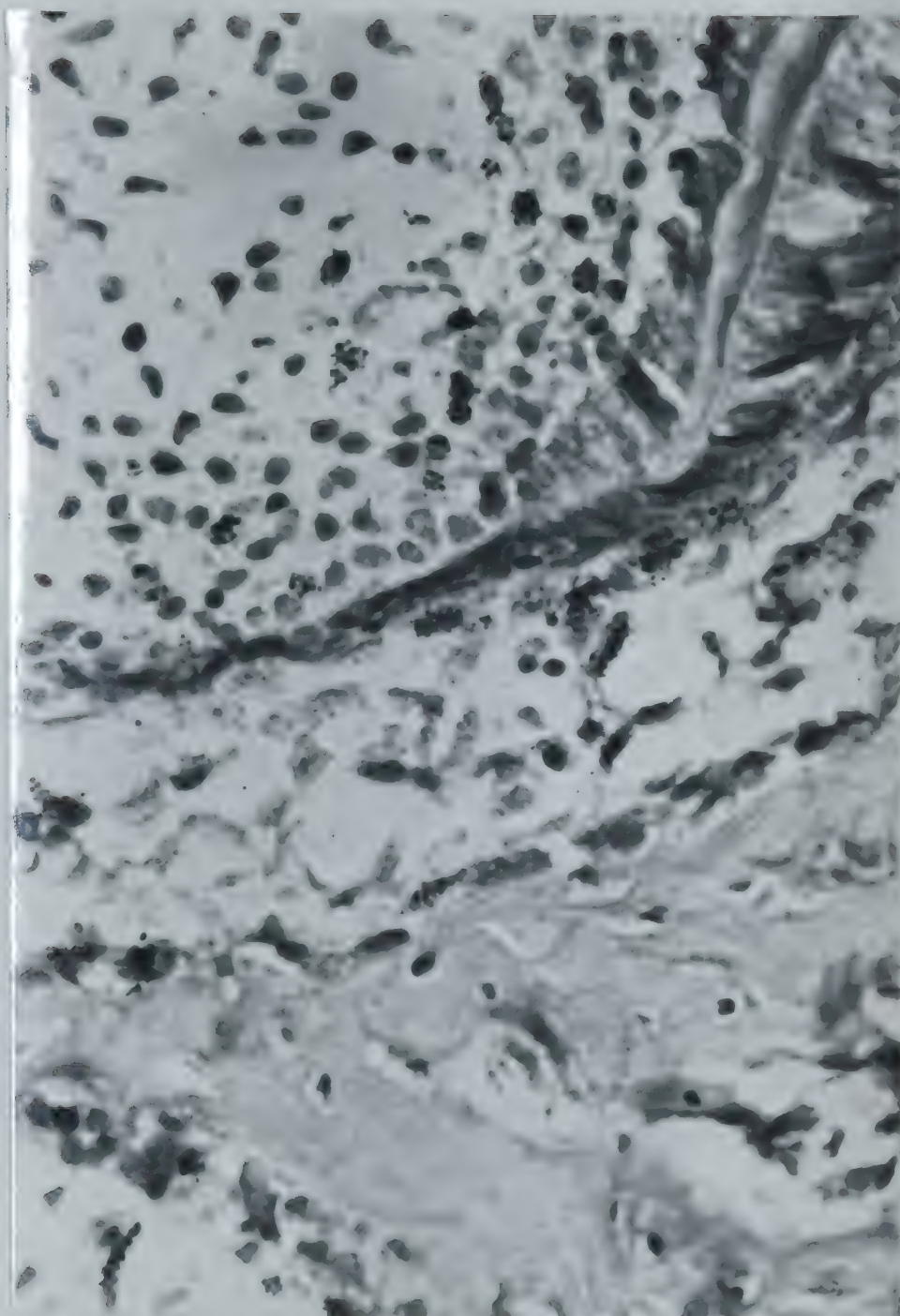


Plate 2 Labelled mouse, showing labelled cells. (x600)

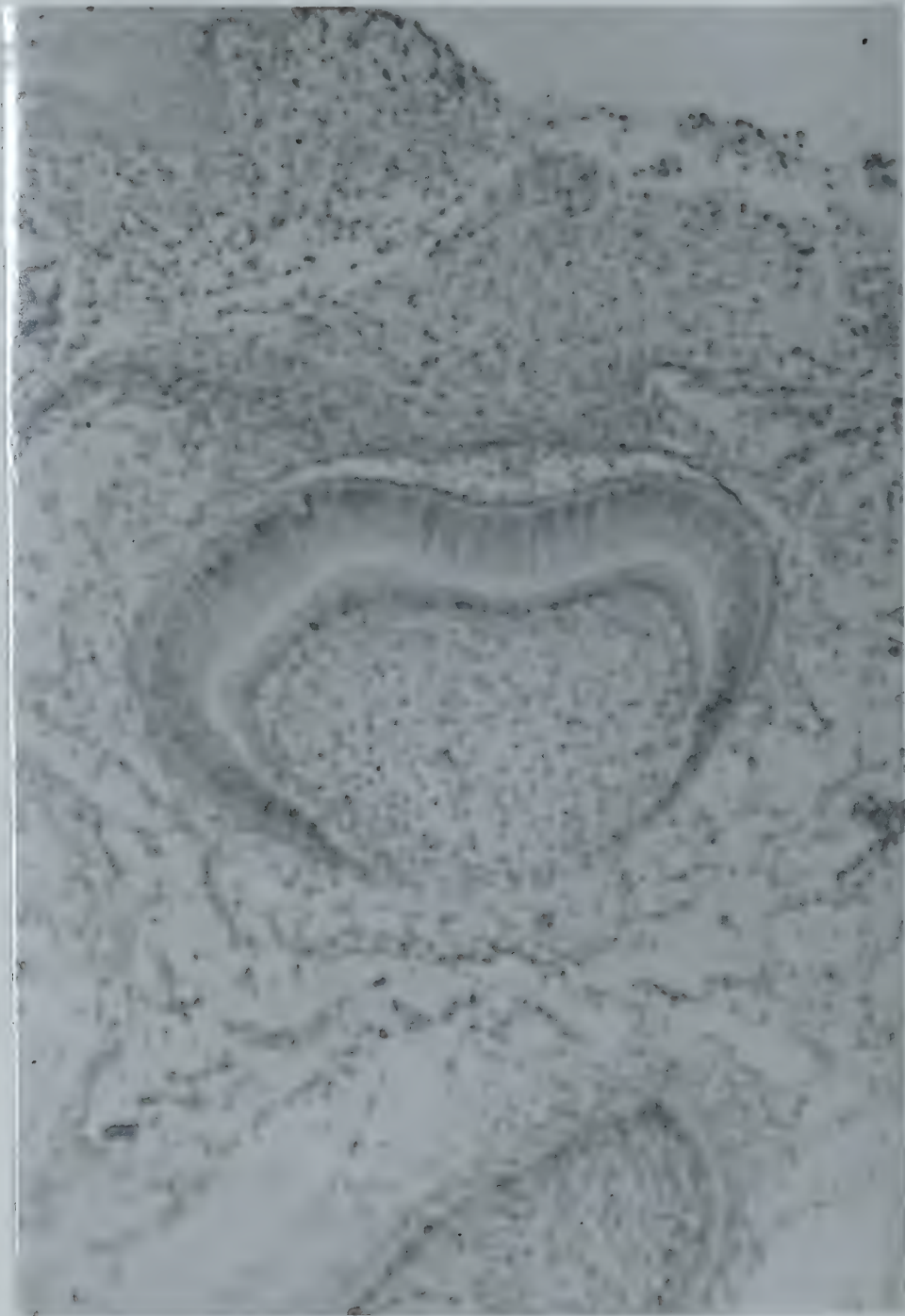


Plate 3 10 day tooth germ. (x200)

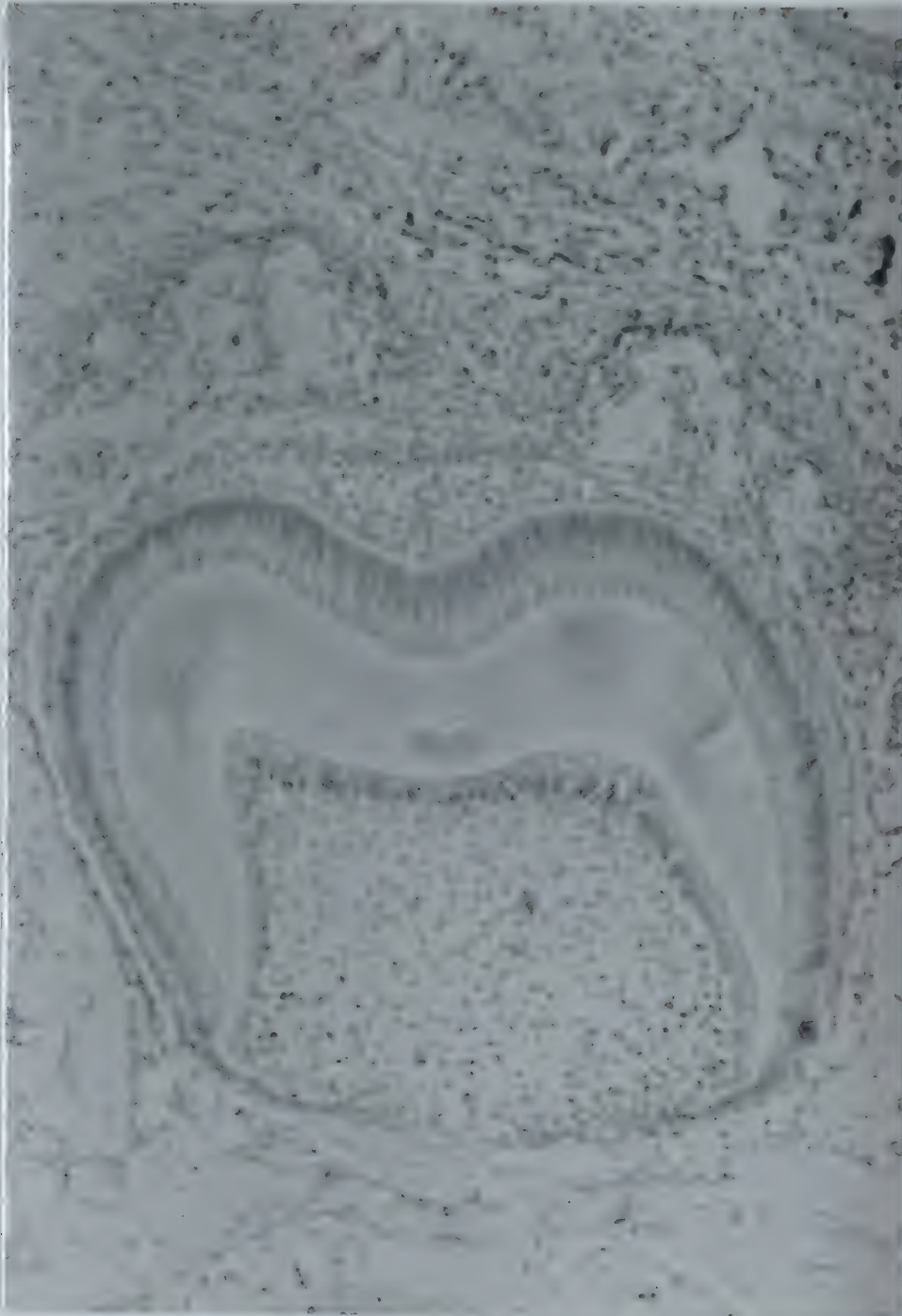


Plate 4 13 day tooth germ. (x200)

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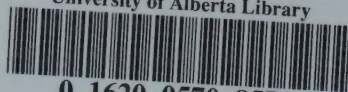
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